STUDY OF TRANSITION METAL THIOBARBITURATE COMPLEXES

BY
JÓZSEF MORVAY

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CONTENTS

1.1. Foreword and objectives

For the analytical investigations of pharmaceutical products a number of reactions are used which are based on complex formation or whose reagents are complexes. Since many of these products contain complexable functional groups, the reactions based on complex formation are well suited for the characterisation and, in many cases, for the determination of the products.

However, several reactions of this type are based only on empirical observations and their chemical mechanisms are unknown. Hence it is desirable to study these reactions in more detail, applying the modern principles of complex chemistry. In several papers (91, 92, 100—103, 122—124) we have already published some of our results in this field, and it seemed to be very important to continue the investigations of these problems. The relation between the effect-mechanism and tendency to complex formation of several pharmaceuticals can be well demonstrated (21, 31). Complex formation may cause absorption, effect-progression or elimination reactions in organisms, and often unexpected processes, too. A number of properties of pharmaceuticals change due to complex formation. In addition to this, catalytic reactions and redox processes must also be taken into account (44, 144). The study of this group of problems can lead to many important results in this field.

Due to their toxicological importance the analytical investigation of barbiturates has been elaborated and seems to be completely concluded. Nevertheless, a number of unstudied problems were found in the case of the thiobarbiturates. The reason for this is probably that, as these drugs are intraveneous narcotics, they are usually applied by experts (anaesthesiologits) in hospitals. Hence toxicosis is extremely rare and the elaboration of their analysis toxicologically has not been of such significance as in the case of the barbiturates.

In this work the most frequently used short-action narcotics, the thiobarbiturates, and their complexes with certain transition metals, were investigated.

The aim was a detailed study of the colour reactions of thiobarbiturates with copper(II) and cobalt(II) ions, the mixed pyridinocopper(II) complexes of thiobarbiturates, and some aspects of their structures. For the purpose of comparative investigations, some N N′ substituted derivatives of thiobarbiturates and also other mixed complexes of pyridino transition metals were prepared.

For the detailed study, the composition analysis of pyridinocopper(II) mixed complexes, their IR absorption and reflection spectra, electrophoretic behaviour, magnetic properties, thermogravimetric data, and microcrystalloscopy were used.

In addition, the photometric determination of thiobarbiturates and the influenc-

ing factors were examined. Several new methods are reported, and it is hoped that the results of theoretical considerations, synthesis and structure investigations obtained so far will be used in practical pharmaceutical analysis.

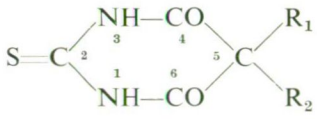
1.2. Introduction

Thiobarbituric acid derivatives, applied intravenously, are modern narcotics. The presence of sulphur in the molecule causes shortening of the period of narcosis, presumably due to lower water- and higher lipoid-solubility. Thiobarbituric acid was first prepared in 1887 by MICHAEL (84, 85), diethyl thiobarbituric acid in 1904 by FISCHER and DILTHEY (40), and different sulphur-containing barbiturates by JOHNSON and HILL (60) in 1911. However, at that time their therapeutical significance was not recognised. TABERN and VOLWILER (139) prepared 5-ethyl-5-(1-methylbutyl)-2-thoibarbituric acid in 1935, and this was successfully applied in therapy in the same year. Use of the thiobarbiturates gradually became widespread. According to BUNDEE (16), their application compared with other intravenous narcotics increased from 0,8% in 1935 to 79,7 per cent in 1954, and at present it is on an even higher level.

Since a number of difficulties arise in the determination of thiobarbituric acids due to their unstability, we attempted to prepare compounds which are more stable and in which the thiobarbiturates can be determined directly. For this purpose, as our preliminary experiments showed, the photometry of copper(II), pyridinocopper(II), and cobalt(II) thiobarbiturate complexes seemed to be the most suitable. Therefore, the preparation of these complexes and the properties of the mixed complexes were examined in detail so that quantitative methods of determination could be devised. In addition, it was planned to study the colour stability of the complexes in different solvents, and the possibility of their separation.

In the course of the investigations, systematic experiments were carried out with the thiobarbituric acid derivatives listed in Table 1, together with some special derivatives reported in Chapter 2.1.

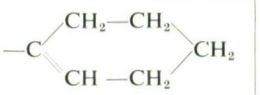
The formula of the parent compound is



where R_1 and R_2=H.

The sodium and thiobarbiturate contents of the thiobarbiturates were determined according to the specifications of the PHARMACOPOEA INTERNATIONALIS, Ed. I, and all the products used met the prescribed requirements (112).

Table 1
The most frequent thiobarbituric acid derivatives subjected to investigation

Chemical name	Commercial name	M. W. g	R_1	R_2	m. p. °C
5—ethyl—5—(1—methylpropyl)—2—thiobarbituric acid	Inactin Brevinarcon Venobarbital	228,30	$-CH_2-CH_3$	$-CH(CH_3)-CH_2-CH_3$	160
5—ethyl—5—(1—methylbutyl)—2—thiobarbituric acid	Thiopental Trapanal Penthotal	242,31	$-CH_2-CH_3$	$-CH(CH_3)-CH_2-CH_2-CH_3$	155
5—allyl—5—(2—methylpropyl)—2—thiobarbituric acid	Baytinal	240,31	$-CH_2-CH=CH_2$	$-CH_2-CH(CH_3)-CH_3$	145
5—allyl—5—(1—cyclohexenyl)—2—thiobarbituric acid	Kemithal Thialbarbiton Intranarcon	264,33	$-CH_2-CH=CH_2$		139
2—thiobarbituric acid		144,15	—H	—H	233

Abbreviations

The chemical names of the thiobarbituric acid derivatives are abbreviated in the text as follows:

5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid	= EMPTB
5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid	= EMBTB
5-allyl-5-(2-methylpropyl)-2-thiobarbituric acid	= AMPTB
5-allyl-5-(1-cyclohexenyl)-2-thiobarbituric acid	= ACHTB
thiobarbituric acid	= TBA
pyridine	= py
barbiturate	= barb
thiobarbiturate	= TB

1.3. Outline of literature

Barbiturates and thiobarbiturates form complexes with different metal ions. LÜDYTENGER (79) prepared the bismuth and iron(III) complexes of barbiturates. BJÖRLING and his co-workers (10, 11), ZAAR and CRONWELL (158), TIMOFEEVA (141), POETHKE and PREUSSLER (113, 114), PFEIL and GOLDBACH (111), and KLOECKING (68) studied the formation and properties of the mercury(II) complex, while FÜRST (46) examined the silver complexes of barbiturates. RISTICI (117) applied thiobarbiturate reagents for the determination of cadmium, then determined the water content of the cadmium(II) thiobarbiturate complex with a derivatograph (118). Nevertheless, most authors used cobalt(II) or copper(II) complexes to determine barbiturates since they can be applied favourably for colorimetric determination of these compounds.

It was LINDBERGER (76) who first studied in 1925 the reaction of barbiturates with copper(II) ions and used the characteristic crystalline "copper compound" of Veronal for toxicological detection. JONSSON (61) investigated the microdetection of barbiturates for example in a copper(II) acetate (potassium acetate) pyridine medium. ZWIKKER (159, 160) prepared the purple-violet precipitate of the pyridinocopper(II) complex of diethylbarbituric acid and determined its composition. His procedure was applied to prepare and determine complexes of different barbituric acid derivatives by a number of authors, e.g. WINTERS (157), WAGENAAR (151, 152), FIELKOV and RAPAPORT (38), LEVI and HUBLEY (75), ROMIJN (119—121) FLOTOW (42) and FÜRST (46.)

According to PARRI's investigations (106), in an ammonia medium or in the presence of an organic base, barbiturates and cobalt(II) chloride form a violet complex which is also suitable for colorimetric determination.

This reaction was later investigated in detail by ZWIKKER (160, 161), KOPPÁNYI and his co-workers (69, 70), BAGGESGAARD—RASMUSSEN and JERSLEV (4), and POPOVA and KRAMORENKO (115). It was also used in various modifications (20, 22, 77, 82, 110) for the determination of certain barbiturates and for the development of chromatograms (43, 131, 143, 153).

Copper(II) and cobalt(II) ions, in the presence of inorganic and organic bases, are especially suitable for the determination of thiobarbiturates due to the *green* colour of their thiobarbiturate complexes. Since barbiturates are purple-violet it is possible to differentiate thiobarbiturates on this basis.

Considerably fewer reactions and modifications can be found for the qualitative

detection of thiobarbiturates than for barbiturates, and there are only sparse data for their determination.

In an investigation of the thiocarbamide derivatives, SHIOMOTANI (132) gave an account of the metal complexes of thiobarbiturates with cobalt and copper salts, and studied their colour formations from a qualitative viewpoint.

SCHMIDT (127) carried out microscopic examinations of crystalline precipitates of many barbiturates and several thiobarbiturates prepared with the ZWIKKER reagent (159) on slides, and established their optical properties. He reported data on Baytinal, Inactin, Thiogenal and Trapanal.

The pyridinocopper(II) reagent was used by JOANID and BORS (59) for the detection of thiobarbiturates in microchemical materials, and by BOUCHERLE and CAU (13) for the same purpose in biological materials.

For the determination of thiobarbiturates, RAVENTOS (11) applied diethylamine and anhydrous copper(II) sulphate in methanol.

In the case of Inactin and Thiogenal, HEISE and KIMBEL (53) suggested an alkaline cobalt(II) chloride solution in methanol.

LOBANOV (78) carried out the determination of 5-ethyl-5-methyl-2-thiobarbituric acid and its sodium salt by means of copper(II) sulphate reduced with hydroxylamine hydrochloride in a solution saturated with ammonia. A yellow complex was produced which could be measured at 400 nm.

It is apparent from this short summary that, in the case of the thiobarbiturates, the cobalt(II) and copper(II) complexes and the mixed complexes formed with amines are the most significant derivatives for the quantitative determination. Therefore, it seemed reasonable to study both of these reactions in more detail, and to examine factors affecting the formation of these complexes and their photometric determination.

GOMAHR and KRESBACH (50) found that all the compounds containing —CO— —NH—CO— or —CO—NH—CS— groups in their molecules gave coloured complexes with cobalt(II) or copper(II) ions and amines. Compuonds containing the —CO —NH—CS— group gave a green colour. As an analogous compound the well-known complex $Cu(II)(py)_2(SCN)_2$ can be mentioned which is also green.

According to AWE and WINKLER (2), all the compounds containing $=N-\overset{II}{C}-N<$ or $-N=\overset{I}{C}-N<$ groups give the ZWIKKER reaction (160). These are as follows: some carbamide and thiocarbamide derivatives such as hydantoin and 5,5-diphenylhydantoin, sulphathiazole, phthalysulphathiazole, succinylsulphathiazole and some other sulphonamides. Of the purine derivatives theobromine, theophylline and thiamine give the reaction. In addition, uracil, alloxan, tricarbonimide, phthalimide and succinimide give similar reactions with barbiturates. However, if the reaction medium is properly chosen these compounds do not disturb the reaction. Hence it can be considered as reasonably specific for the thiobarbiturates.

The PARRI (106) and ZWIKKER (159) reactions have been investigated many times in connection with barbiturates and the results can be summarized as follows.

In the PARRI reaction, absolute ethanol, chloroform, ethyl acetate and glycerin have been used besides methanol. Methanol and chloroform proved to be the most suitable.

Cobalt(II) was applied as its nitrate, chloride of acetate.

To make the medium alkaline, ammonium, potassium, sodium, lithium and barium hydroxides have been applied. According to ZWIKKER's examinations (159), in the case of lithium hydroxide, the sensitivity of the reaction decreases significantly.

Instead of these inorganic compounds, diethylamine, n- and isopropylamine,

n- and isobutylamine, isoamylamine, pyridine, piperidine and piperazine were suggested to produce the basic medium.

KOPPÁNYI and his associates (69, 70) established that the saturated primary amines, especially isopropylamine, gave the best results. In an examination of barbiturates, ZWIKKER (159) suggested the use of pyridine together with copper(II) because it was found that pyridine increased the colour intensity and the sensitivity of the reaction.

The validity of the Bouguer-Lambert-Beer rule for barbiturates in these reactions, disputed by some authors, was proved by PAULUS and PRIBILLA (110). They found the peaks of the absorption curve to lie between 560 and 600 nm depending on the reaction conditions.

The cooper(II) and cobalt(II) complexes of barbiturates and thiobarbiturates also show a characteristic absorption in the UV region. These bands can be assigned to the barbiturate and thiobarbiturate components (80).

The UV spectra of the barbiturate derivatives were investigated by POETHKE and PREUSSLER (114) and STANOVNIK and TISLER (135).

Contrary to the opinion of DILLE and KOPPÁNYI (28), BRUNDAGE and GRUBER (15) found that there was no connection between the intensity of the colour produced by complex formation and the molecular weight of barbiturates. Consequently it was impossible to use one barbiturate as standard for the determination of another one, and to convert the result by a factor as in the case of sulphonamides.

There have been a number of hypotheses as to the composition of the complexes. ZWIKKER (160) described the following barbiturate complexes:

$[(barb)_2\ Co\ (NH_3)_6]OH$	brown
$[(barb)_2\ Co\ (NH_3)_5]OH$	brown
$[(barb)_2\ Co\ (ClOH)]K$	blue
$[Cl_2(barb)_2\ Co]Ba.3[Cl(OH)(barb)_2\ Co]Ba$	violet
$[(barb)_2\ Cu\ (py)_2]$	violet

In anhydrous methanol containing potassium hydroxide the resulting compound proved to be $Co(barb)_2$.

The reaction between 5-dialkylated and N-methylated barbituric acids [prepared by BODENDORF's modification (12)] cobalt(II) salts and ammonium hydroxide was examined by SCHWENKER (130) who found that the composition of the complex was as follows:

$$Co(NH_3)_2(barb)_2$$

A coordination complex is formed when the alkaline component is ammonia or an organic base.

These points will be discussed later.

To summarize, the PARRI reaction is frequently used for the determination of barbiturates although it is not quite specific. Among its numerous advantages should be mentioned its simplicity and rapidity. There are also some disadvantages, e. g. it is not too sensitive, in the presence of inorganic hydroxides the colour is stable for only a limited time, and the colour intensity is not proportional to the molecular weight.

The ZWIKKER reaction is more specific; it does not react with purine derivatives but does react with saccharin.

No such data can be found for the determination of thiobarbiturates. Complexes

formed from copper(II) are more stable than those obtained from cobalt(II) ions, so they are more suitable for colorimetric determinations.

Since these findings were verified in the preliminary experiments, the first problem was to examine the pyridinocopper(II) thiobarbiturate complexes in detail.

A number of Hungarian authors have dealt with the analysis of barbiturates, in particular BAYER and POZSGAI (6), BURGER, SCHULEK and LADÁNYI (17), CSÁSZÁR and SZÜCS (25), CZUCZY (26), DÁVID (27), EKKERT (33, 34), FUCHS and coworkers (45), GERVAY (47), GYENES (52), Mrs. MINCSEV (87—89), NIKOLICS (105). SCHULEK and RÓZSA (129), SZÉP and GRUSZ (138), SZÁSZ (137), SZABOLCS (147), VÉGH (150), WESSEL and KRESZLER (155).

The decomposition of thiobarbiturate solutions was studied by VASTAGH and SZABOLCS (147), Mrs. VASTAGH and VASTAGH (148), and Mrs. VASTAGH (149).

2.0. Investigation of transition metal pyridine thiobarbiturate mixed complexes

2. 1. Preparation of thiobarbiturate compounds used in the investigation

The thiobarbituric acids in Table 1 were prepared by precipitation from the corresponding commercially available sodium salts with hydrochloric acid in aqueous medium. After filtration and recrystallization from aqueous ethanol their melting points were checked. The acids are required rather than the sodium salts because the latter, used for intraveneous injections, easily decompose when the ampoule is opened, they are not stable in solution and hence must be treated with care.

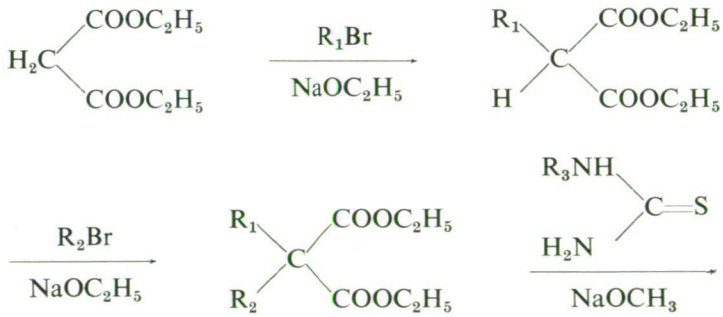
5,5′-substituted thiobarbituric acids were obtained simply as above.

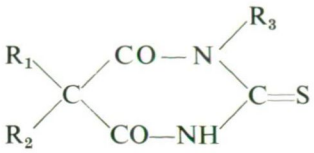
However, the hydroxy derivatives of thiobarbituric acid were required and, also for comparative purposes, the 5-, N-, 5,N-, N,N′-, 5,5′,N-, 5,N,N′-, and 5,5′,N,N′-substituted derivatives. There are examples in the literature of the synthesis of each type of compound. Nevertheless, it appears reasonable to publish our syntheses and analytical results on the basis of uniformity.

In addition to those mentioned in Chapter 1.2. (40, 60, 84, 85, 139), several authors have prepared thiobarbituric acid and its derivatives: EINHORN (32), WHEELER and JAMIESON (153), MILLER, MUNCH, CROSSLEY and HARTUNG (86), HEPNER and FRENKENBERG (54), CROSSLEY, MILLER, HARTUNG and MOORE (24), DOLZE, GOLDHAHN and FÜRST (29), and SALVESEN (125).

For the preparation of the derivatives in question we followed essentially the method of FISCHER and DILTHEY (40).

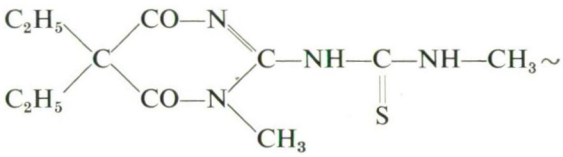
The basic synthesis equation is:

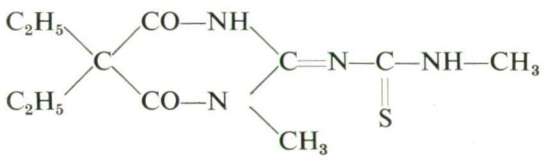




Condensation of malonic acid esters with thiocarbamide takes place in a relatively simple way, and the yield is better than in the case of barbiturates; this is particularly so in anhydrous methanolic media. Unsubstituted and monoalkylmalonic esters condense with N-alkyl- or N,N′-dialkylthiocarbamide similarly with a satisfactory yield. However, condensation of a dialkylmalonic ester with N-methyl- or N-ethylthiocarbamide results in a mixture, and the expected trisubstituted thiobarbituric acid is not the main product.

HESSE et al. (55) have pointed out that the main product is the 2-iminobarbituric acid. From the reaction mixture of diethylmalonic acid diethyl ester and N-methylthiocarbamide, in addition to the expected 5,5′-diethyl-1-methyl-2-thiobarbituric acid, the following compound was isolated:





As a result of suitably chosen reagents and reaction conditions we succeeded in preparing the expected trisubstituted thiobarbituric acid in a satisfactory amount.

For the preparation of 5,5′-dialkyl-1-methyl-2-thiobarbituric acid the most favourable conditions were ensured by the following reagent ratio: 1,5 mole of the substituted diethyl malonate, 1 mole of methylthiocarbamide, and 1,1 mole of sodium dissolved in methanol.

1,5 dialkyl- and 1,3,5,5′-tetraalkylthiobarbituric acids do not react with N-alkylthiocarbamide in alkaline solution. This explains the observation that the synthesis of N-unsubstituted 2-thiobarbituric acid is followed by that of 5-alkyl-N-alkyl-2-thiobarbituric acid and 5,5′,N,N′-tetraalkyl-2-thiobarbituric acid and the product is the expected corresponding 2-thiobarbituric acid.

It was reasonable to discuss all this in detail because the 2-iminobarbituric acid derivative, possibly present as an impurity, might disturb the complex formation reactions.

Experimental

(Melting points are not corrected.)

Starting materials were either obtained from PROMONTA GmbH or were synthesized.

The following model compounds were prepared:

1.) *2-Thiobarbituric acid.* 0.6 mole of sodium was dissolved in 240 ml of anhydrous methanol and the solution cooled to room temperature. 0.32 mole of thiocarbamide (dried at 100 °C) and 0.2 mole of diethyl malonate were added with continous stirring which was continued for another hour at room temperature. The mixture was refluxed for 7 hours (still with stirring), concentrated to one third of its volume, and diluted with 100 ml of water. The resulting 2-thiobarbituric acid was precipitated by the addition of 5N hydrochloric acid until the mixture became acidic. The product was dried and repeatedly recrystallized from ethanol/water.

Yield: 61%
Molecular weight: 144.15
M. p.: 233—5 °C (decomp.)

Analysis:

	C %	H %	N %	S %	O %
Calculated	33.33	2.79	19.46	22.25	32.20
Found	33.26	2.85	19.40	22.17	—

Preparation of 1-alkyl-, 5-alkyl-, 1,3-dialkyl-, 1,5-dialkyl-, and 1,3,5-trialkylthiobarbituric acids

The preparation of these compounds is similar to that described for thiobarbituric acid. Unsubstituted or monoalkyl-substituted malonic ester was condensed with thiocarbamide, N-alkylthiocarbamide or N,N′-dialkylthiocarbamide. These thiobarbituric acids are stronger than the 5,5′-disubstituted acids; therefore, it was possible to purify them as follows.

The crude product was repeatedly recrystallized from ethanol/water, and dissolved in ethanol by boiling. 20% sodium hydroxide solution was added dropwise until the solution became neutral. Monosodium salts of thiobarbituric acids were crystallized; for analytical purposes the corresponding thiobarbituric acid was obtained from the latter with diluted hydrochloric acid.

2) *1-methyl-2-thiobarbituric acid*

Yield: 82%
Molecular weight: 158.1
M. p.: 201—2 °C

Analysis:

	C %	H %	N %	S %	O %
Calculated	38.00	3.80	17.72	20.29	20.24
Found	37.87	3.70	17.59	20.18	—

3) *1, 3-diethyl-2-thiobarbituric acid*

Yield: 73%
Molecular weight: 200.2
M. p.: 112—3 °C

Analysis:

	C %	H %	N %	S %	O %
Calculated	47.99	6.03	13.99	16.01	16.00
Found	47.82	6.13	13.93	16.08	—

4) *1-methyl-5-phenyl-2-thiobarbituric acid*

Yield: 51%
Molecular weight: 234.2
M. p.: 282—3 °C

Analysis:

	C %	H %	N %	S %	O %
Calculated	56.39	4.3	11.95	13.69	13.67
Found	56.27	4.5	11.87	13.60	—

5) *1,3-diethyl-5-phenyl-2-thiobarbituric acid*

Yield: 6%
Molecular weight: 276.3
M. p.: 162—3°C

Analysis:

	C %	H %	N %	S %	O %
Calculated	60.85	5.83	10.13	11.60	11.59
Found	60.68	5.90	10.08	11.45	—

Preparation of 1,5,5'-trialkyl-2-thiobarbituric acid derivatives

6.) *5,5-diethyl-1-methyl-2-thiobarbituric acid.* 2.5 g (0.11 mole) of sodium was dissolved in 50 ml of methanol, the solution cooled to room temperature, and 9 g (0,1 mole) of N-methyl-2-thiocarbamide (dried at 100 °C) and 32.5 g (0.15 mole) of diethyl malonate (b. p. 106 °C) were added with continuous strirring. The mixture was stirred for half an hour at room temperature, gently refluxed (with stirring) for 10 hours, concentrated to one third of its volume by distillation, and then 100 ml of water were added. The residual ester was extracted from the mixture with benzene, and the barbituric acid was precipitated from the aqueous phase by adjusting the latter to pH 2 with 5N hydrochloric acid. The organic acid was reextracted with benzene, which was then washed with a saturated aqueous solution of $NaHCO_3$ to remove any malonic acid. The purified benzene solution was extracted with 1 N NaOH from which the thiobarbituric acid was precipitated with hydrochloric acid. It was allowed to crystallize in a refrigerator. After filtration it was separated from any 5,5'-diethyl-1,2-methyl-thiocarbonyl-iminobarbituric acid by treatment with carbon tetrachloride; the latter derivative is insoluble but thiobarbituric acid readily dissolves.

Pure thiobarbituric acid was obtained by recrystallization from ethanol/water.
Yield: 9%
Molecular weight: 214.2
M. p.: 79.5 °C

Analysis

Calculated	C % 50.44	H % 6.58	N % 13.00	S % 14.97	O % 11.96
Found	50,32	6.60	12.92	14.85	—

Preparation of tetraalkyl-2-thiobarbiturates

7.) *1,3,5,'-tetraethyl-2-thiobarbituric acid.* 6.9 g (0.3 mole) of sodium was dissolved in 120 ml of anhydrous methanol and 22 g (0.16 mole) of N,N'-diethyl-thiocarbamide (dried over P_2O_5 in vacuum) and 21.6 g (0.1 mole) of diethylmalonic acid diethyl ester (b. p. 106 °C) were added with continuous stirring. The mixture was refluxed (with stirring) for 10 hours, concentrated to one third of its volume, and cooled. 100 ml of water was added, followed by 5N hydrochloric acid to pH 2, when the 5,5',1,3-tetraethyl-2-thiobarbituric acid separated out as an oil. It was extracted with ether, the extracts dried with sodium sulphate, and the ether distilled off. The resulting oil was fractionated under reduced pressure. Sheet crystals separated from the distillate; these were dried and recrystallized from ethanol.

Yield: 4%
Molecular weight: 256.35
M. p.: 75 °C

Analysis:

Calculated	C % 56,22	N % 7,86	N % 10,92	S % 12,51	O % 12,50
Found	56,08	7,72	10,85	12,39	—

2.1.1. Some data on the formation of pyridinocopper(II) complexes with different thiobarbiturate derivatives not used as pharmaceuticals

It was reported by BAGGESGAARD-RASMUSSEN and JERSLEV (4) that 5,5- and 5,5,N-substituted barbituric acid derivatives, in a concentration of 0.005—0.25%, can be determined with cobalt(II) chloride and isobutylamine in chloroform. The 5-, N-, 5,N-, N,N'-, 5,N,N'-, and 5,5,N,N'-substituted derivatives do not give colour reactions and thus their quantitative determination is not possible in this way. The same fact is true for barbituric acid itself.

These data are valuable since they prove that the central metal atom can give a colour reaction only if a hydrogen atom remains unsubstituted on at least one of the N atoms of the pyrimidine ring, and if two substituents are present on C-5.

In compounds where these requirements are not met, it is not possible to obtain the enol form of barbiturates necessary for bonding with the metal ion.

In the case of thiobarbiturates such data could not be found and, therefore, we carried out the PARRI and the ZWIKKER reactions according to Chapter 2.2.

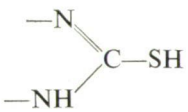
with the 5-, N-, 5,N-, N,N′-, 5,N,N′-, and 5,5,N,N′-substituted thiobarbituric acids which had been prepared according to Chapter 2.1. We found that neither pyridinocobalt(II) nor pyridinocopper(II) complexes could be formed. The same is also partially true for barbituric acid itself. Our results are generalized and summarized in Table 2.

Table 2

Reactions of thiobarbiturate derivatives with pyridinocopper(II) (ZWIKKER) and pyridinocobalt (II) (modified PARRI) reagents

Positive; green colour formation	No reaction
5,5'-substituted-2-TB	5-substituted-2-TB
5,5',N-substituted-2-TB	N-substituted-2-TB
	N,5-substituted-2-TB
	N,N'-substituted-2-TB
	5,N,N'-substituted-2-TB
	5,5',N,N'-substituted-2-TB
	2-TB

The behaviour of N,N′-substituted thiobarbituric acid derivatives proves unambiguously that if there is no way to form a



group no complex formation takes place. This fact supports at the same time the hypothesis that the transition metal — TB bond is formed with the participation of the sulphur atom.

The N-hydrogen of thiobarbiturates is activated due to its tendency to conjugation and, in the presence of substituents of positive inductive effect on C-5, as a consequence of the electron releasing effect of these substituents. It is, therefore, inclined to form a sulphohydryl (en-thiol) group on C-2 and to give complexes.

Our experiments clearly show that the two substituents on C-5 play an important role.

It is very interesting to compare these data with the pharmacological results. Investigation shows that no narcotic effect occurs unless there are at least two substituents on C-5, and that these should not be methyl but some of its higher homologues or other groups. The pyrimidine ring is activated by N-substitution and these compounds easily decompose in the body and become ineffective. The N,N′-substituted derivatives are toxic. Thiobarbituric acid derivatives decompose more easily than the corresponding barbituric acid derivatives, and consequently they are the best short-effect narcotics.

In agreement with the finding of ZWIKKER (159) who examined the PARRI reaction in the case of the cobalt(II) complex, the pyridinocopper(II) thiobarbiturate complexes also easily decompose with mineral acids. The resulting thiobarbituric acid can be extracted with ether and recovered unchanged.

These investigations also support the supposition that transition metal complexes are not formed with decomposition products of thiobarbiturates or with the possible toxic N,N'-substituted impurity. This is important from an analytical respect.

2.2. Preparation of pyridino thiobarbiturate mixed complexes of copper(II), cobalt(II), cadmium(II) and nickel(II)

For our examinations we had to prepare pyridino transition metal complexes of some thiobarbiturates in crystalline from. As no literature data were available, we attempted to produce the above compounds according to the methods of FIELKOV and RAPAPORT (38), FÜRST (46) and LEVI and HUBLEY (75) originally applied for the preparation of pyridinocopper(II) complexes of barbiturates. However, these methods did not give the desired results since their simple transfer to the thiobarbituric acid derivatives proved to be unsuccessful. Therefore, we first performed some studies to determine the quantitative ratio of thiobarbiturate and pyridinocopper(II) reagent required for the preparation of the complex.

Thiobarbituric acid and its derivatives are insoluble in water. When preparing a 2×10^{-2} M stock solution, sufficient pyridine was added to give a pyridine:water v/v ratio of 40:60. As reagent an aqueous pyridine solution of copper(II) sulphate was used containing 5×10^{-2} M copper(II) sulphate. Before completing to volume 100, 150, 200 or 250 ml of pyridine was added (150 ml of pyridine proved to be most suitable). In this way we obtained an aqueous solution containing 15% pyridine which was enough for the formation of $Cu(II)(py)_3$ ($K_3=1.14$) and $Cu(II)(py)_4$ ($K_4=0.6$) complexes, and to establish a pH of 7.4. The colour change of the solution to deep blue clearly indicated the complex formation. This reaction could also be followed photometrically after addition of increasing quantities of pyridine by the mole-ratio method. In the following text this compound is called the pyridinocopper(II) reagent.

2.0 ml of the stock solution was allowed to react with different quantities of the pyridinocopper(II) reagent, and the degree of crystallization and the colour of crystals were observed. After filtration the crystals were washed with 5×8 ml of water and 5×8 ml of 10% methanol/water, and dried over concentrated sulphuric acid phosphorus pentoxide in a vacuum desiccator.

The results are summarized in Table 3.

The results of the above investigations show that by adding 0.4—0.5 ml of pyridinocopper(II) reagent to 2 ml of 2×10^{-2} M stock solution of the thiobarbituric acid derivatives examined, crystals form within an hour. If the quantity of reagent is decreased, the crystal-yield also decreases, until finally no crystals are obtained. The same can be observed by increasing the quantity of reagent, considerably probably due to the dissolution of the precipitate.

If a little water is poured into the reaction mixture, the solution becomes turbid and a precipitate is obtained; this assumes its characteristic crystal form presented in Chapter 2.3. only after long standing.

Table 3

Preparation of pyridinocopper(II) thiobarbit urate complexes in crystalline form

2×10^{-2} M thiobarbiturate 2 ml	Pyridinocopper(II) reagent ml	Crystallization	Colour	Time passed till the observation (min.)
EMPTB	0,3	+++	greenish-brown	60
	0,5	+++		60
	0,8	+		180
	1,2	—		—
	2,0	—		—
EMBTB	0,3	+++	greenish-brown	60
	0,5	+++		60
	0,8	+		120
	1,2	—		—
	2,0	—		—
AMPTB	0,3	+++	greenish-brown	60
	0,5	+++		60
	0,8	++		90
	1,2	+		120
	2,0	+		120
ACHTB	0,3	++	pale violet	60
	0,5	++	green	60
	0,8	++	green	60
	1,2	+	green	90
	2,0	+	green	120

Note: +++ abundant
++ moderate
+ slight

In the case of ACHTB with a small amount of reagent, the precipitate is pale violet. If the amount of reagent added is increased to 0,5 ml, the colour of the precipitate becomes green.

Starting with the sodium salts of thiobarbituric acid derivatives, the preparation of the crystalline form of the complex was not successful. However, the colour of the solutions of these complexes is on intense dark green which is constant for a long time.

The complexes are soluble in dimethylformamide, pyridine, chloroform, benzene and methanol, but insoluble in water.

For the investigations, copper(II) complexes were prepared in larger quantities: 0.53 g of ACHTB, 0.48 g of EMBTB, 0,48 g of AMPTB or 0.45 g of EMPTB were dissolved in 40 ml of pyridine, then 60 ml water was added; to this solution 25 ml of pyridinocopper(II) reagent was added in a thin stream with continuous stirring.

In the case of pyridinocopper(II)-ACHTB and pyridinocopper(II)-EMBTB the precipitates are formed at once. Pyridinocopper(II)-AMPTB and pyridinocopper(II)-EMPTB give dark olive-green solutions which become turbid on the addition of a little water. Precipitation takes place if the solutions stand overnight in a refrigerator.

The pH values of the mother liquors were 7.0—7.3. After filtration, washing, and drying, the precipitates were weighed.

Yield:

pyridinocopper(II)-ACHTB	0.66 g	88%
pyridinocopper(II)-AMPTB	0.61 g	87%
pyridinocopper(II)-EMPTB	0.47 g	71.2%
pyridinocopper(II)-EMBTB	0.59 g	84.2%

After the preparation of the pyridinocopper(II) thiobarbiturate complexes, it was attempted to prepare the crystalline pyridino thiobarbiturate mixed complexes of cobalt(II), nickel(II), cadmium(II) and manganese(II). The preparation was carried out as described above.

As reagents, the following 5×10^{-2} M solutions were made: cobalt(II) nitrate nickel(II) sulphate, cadmium(II) nitrate or manganese(II) chloride in water containing 15% pyridine. From these reagents 0.4 ml and 0.8 ml were added, respectively, to 2 ml portions of 0.02 M thiobarbiturate stock solutions. Crystal formation occurs on the addition of as little as 0.4 ml of the pyridinocobalt(II), nickel(II), and cadmium (II) reagents, but no crystals form with the pyridinomanganese(II) reagent.

Our results are shown in Table 4.

Table 4

Formation of pyridinocobalt(II), cadmium(II), and nickel(II) thiobarbiturate complexes

Investigated compound	2×10^{-2} M TB ml	Pyridinocobalt (II) reagent ml	Crystal formation	Colour
EMPTB	2	0,4	++	brown
AMPTB	2	0,4	+	brown
EMBTB	2	0,4	+	brown
ACHTB	2	0,4	+	brown
		Pyridinocadmium(II) reagent ml		
EMPTB	2	0,4	—	—
		0,6	—	—
AMPTB	2	0,4	+	colourless
EMBTB	2	0,4	+	colourless
ACHTB	2	0,4	+	colourless
		Pyridinonickel(II) reagent ml		
EMPTB	2	0,4	++	green
AMPTB	2	0,4	++	pale green
EMBTB	2	0,4	++	dirty green
ACHTB	2	0,4	++	green

Note: + very slight
++ adequate

That is the separation of the complexes in crystalline form took place in every case except that of the pyridinocadmium(II)-EMBTB complex. The pyridinocobalt(II) reagent gave a satisfactory amount of crystals in 8 hours with EMPTB, whereas with AMPTB, EMBTB and ACHTB a longer standing (about 48 hours) was necessary. The separation is also slow when using the pyridinocadmium(II) reagent, but with the pyridinonickel(II) reagent crystal formation takes place within 2 hours.

The crystal formation of ACHTB seems favourable with the above reagents; under the microscope characteristic crystals could be observed.

In addition to the pyridinocopper(II) thiobarbiturate mixed complexes, the pyridinocobalt(II) thiobarbiturate mixed complexes were prepared in larger amount as described above. The difference was that 30 ml of the corresponding reagent was added to the 2×10^{-2} M pyridine/water solution (40:60) of the thiobarbiturates. On adding a little water the solution became turbid and, after standing in a refrigerator overnight, a brownish microcrystalline precipitate formed. After filtration, washing and drying the precipitate was weighed.

Yield:

pyridinocobalt(II)-ACHTB	0.49 g	66.2%
pyridinocobalt(II)-AMPTB	0.45 g	65.2%
pyridinocobalt(II)-EMPTB	0.56 g	84.8%
pyridinocobalt(II)-EMBTB	0.38 g	55.0%

2.3. Microscopic appearance of pyridinocopper(II), cobalt(II), cadmium(II) and nickel(II) thiobarbiturate mixed complexes

According to CALAS and MARTINEZ (18) the barbiturates form monoclinic crystals. The investigation of the mixed complexes described in Chapter 2.2. showed that pyridinocopper(II) thiobarbiturate complexes were greenish-brown under the microscope. The pyridinocobalt(II) thiobarbiturate is brown, the pyridinonickel(II) thiobarbiturate is greenish-yellow, and the pyridinocadmium(II) thiobarbiturate is colourless.

Of the prepared complexes the pyridinocopper(II) complexes of EMPTB, EMBTB, and AMPTB were examined from a toxicological aspect under the microscope by SCHMIDT (127) using another method of preparation.

The crystalline form and microscopic appearance of the complexes are as follows.

It may be seen from the Figures that the thiobarbiturates form differently-shaped crystal complexes with the different transition metals and pyridine; therefore, this fact can be used to distinguish them.

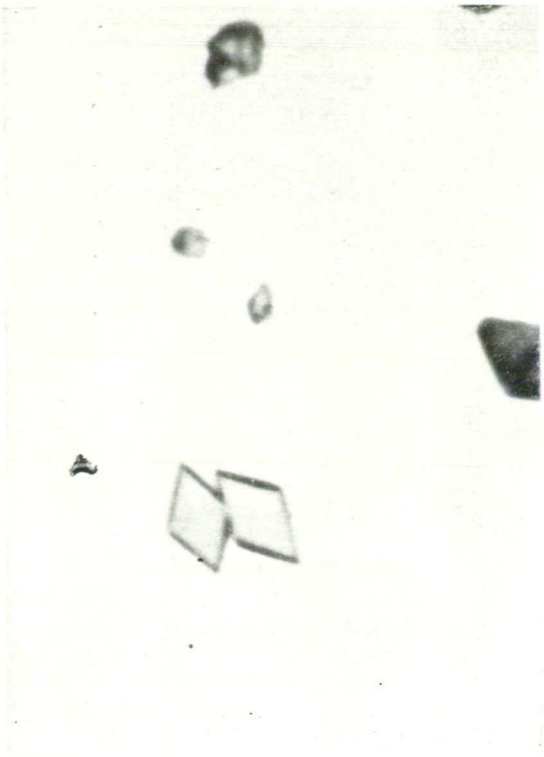


Fig. 1: Pyridinocopper(II)-EMPTB complex.
Magnified 220 times.

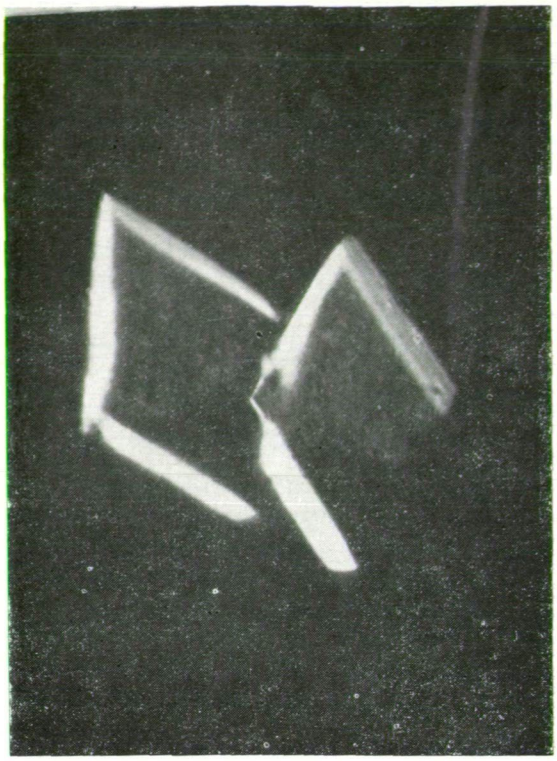


Fig. 2: Pyridinocopper(II)-EMPTB complex.
Magnified 680 times.

Fig. 3: Pyridinocopper(II)-EMPTB complex.
Magnified 170 times.

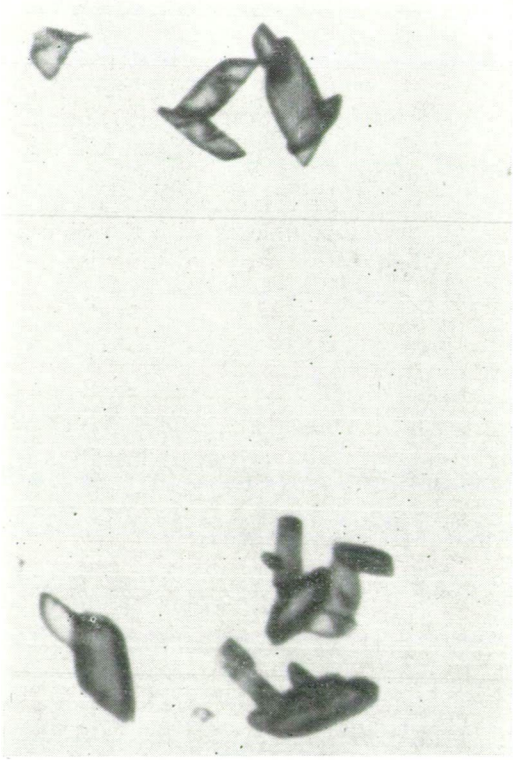


Fig. 4: Pyridinocopper(II)-AMPTB complex.
Magnified 170 times.

Fig. 5: Pyridinocopper(II)-ACHTB complex.
Magnified 270 times.

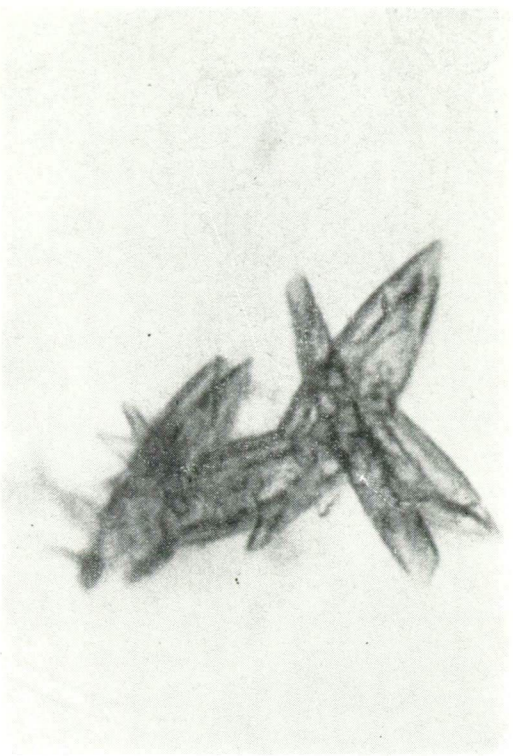


Fig. 6: Pyridinocopper(II)-EMBTB complex.
Magnified 680 times.

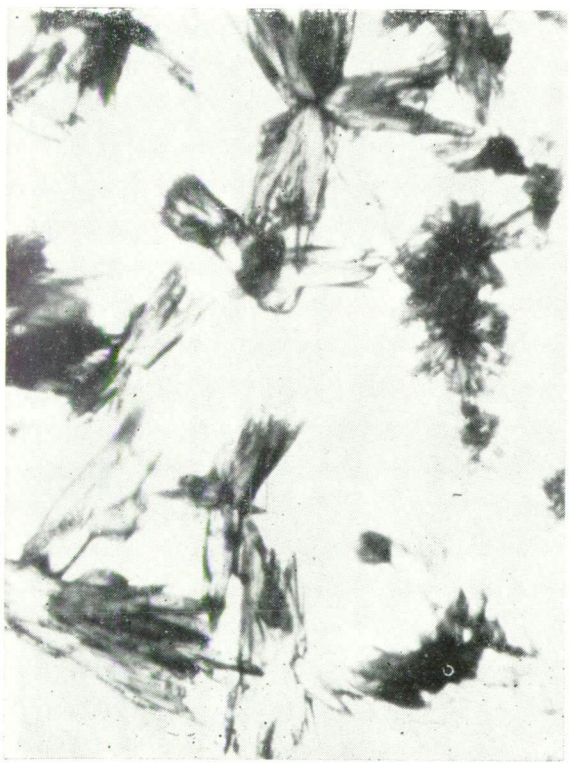


Fig. 7: Pyridinocobalt(II)-EMPTB complex.
Magnified 510 times.

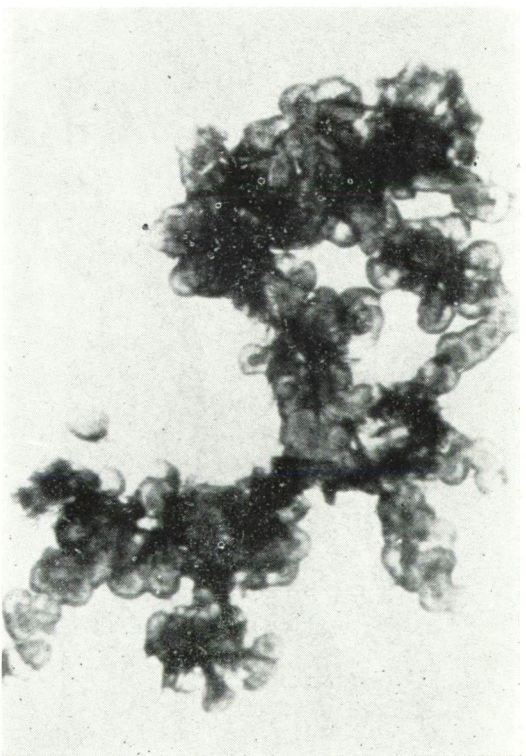


Fig. 8: Pyridinocobalt(II)-EMBTB complex.
Magnified 510 times.

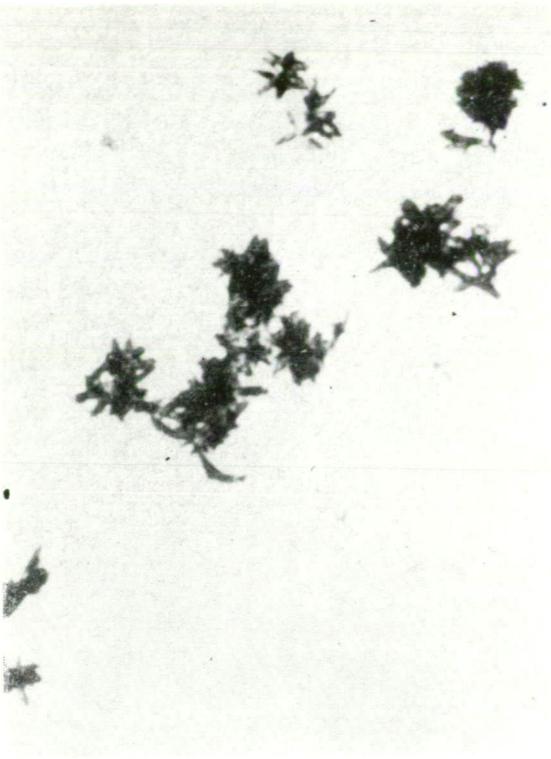


Fig. 9: Pyridinocobalt(II)-ACHTB complex.
Magnified 170 times.

Fig. 10: Pyridinocobalt(II)-AMPTB complex.
Magnified 510 times.

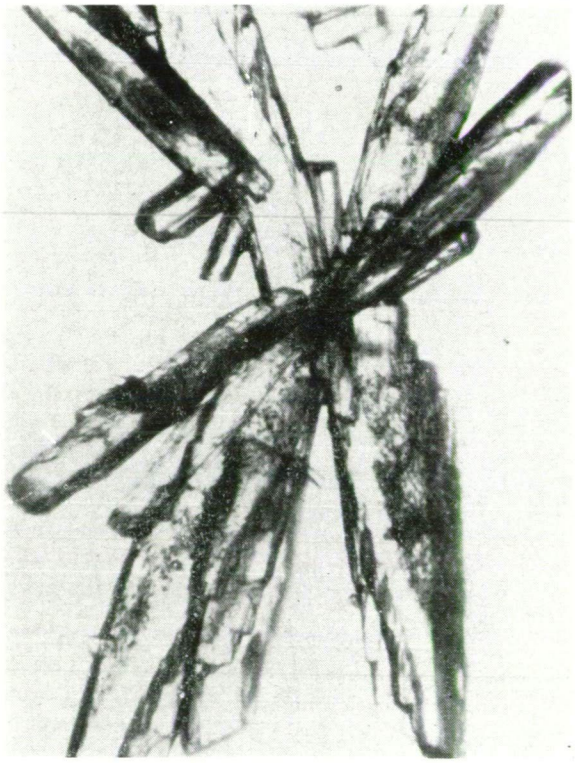


Fig. 11: Pyridinocadmium(II)-EMBTB complex.
Magnified 510 times.

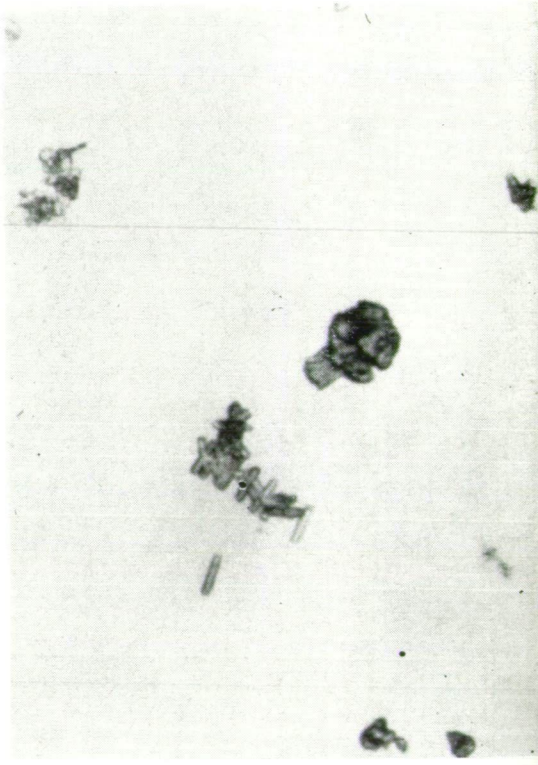


Fig. 12: Pyridinocadmium(II)-ACHTB complex.
Magnified 170 times.

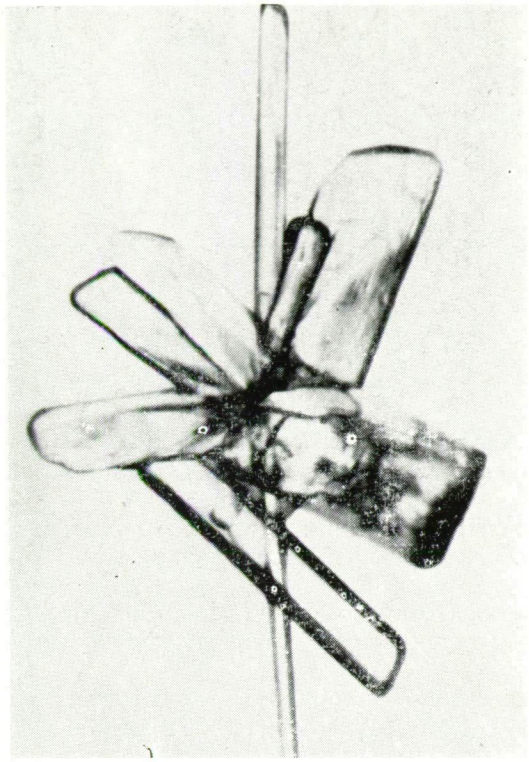


Fig. 13: Pyridinocadmium(II)-AMPTB complex.
Magnified 510 times.

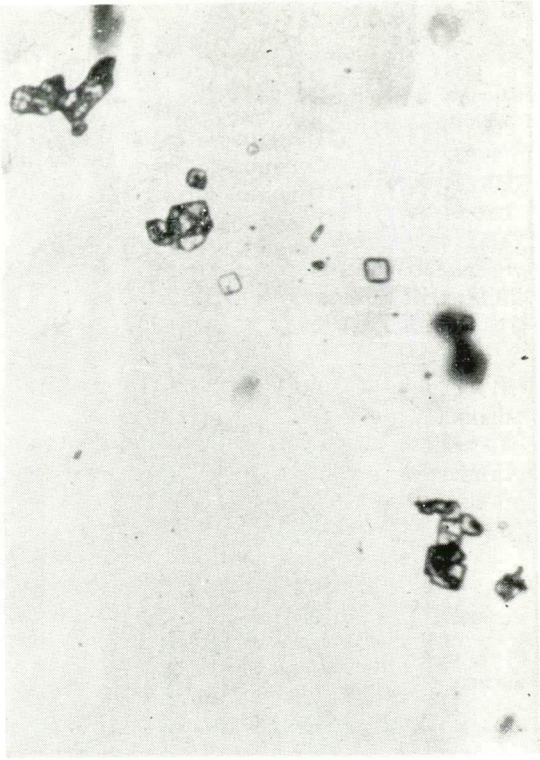


Fig. 14: Pyridinonickel(II)-AMPTB complex.
Magnified 510 times.

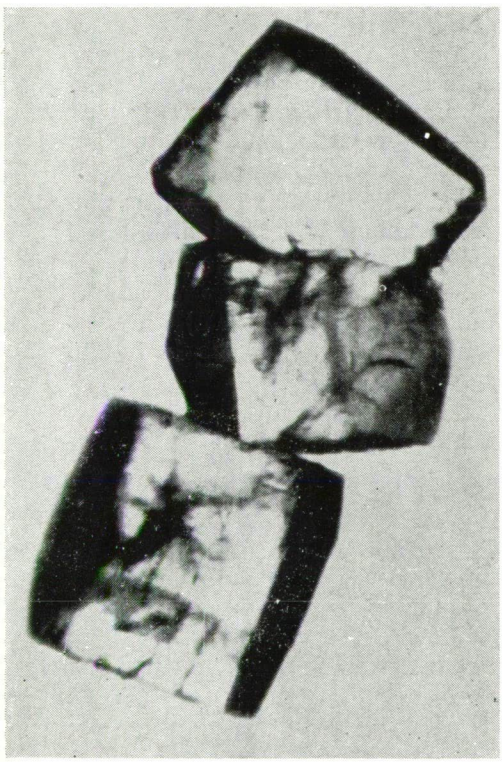


Fig. 15: Pyridinonickel(II)-EMPTB complex.
Magnified 510 times.

Fig. 16: Pyridinonickel(II)-EMBTB complex.
Magnified 510 times.

2.4. Determination of the composition of pyridinocopper(II) thiobarbiturate mixed complexes

To establish the composition of pyridinocopper(II) thiobarbiturate mixed complexes, their C, H, N, S and Cu contents were determined. Their melting points were also taken.

Sulphur was first determined as sulphate by the method of WINKLER (36) after ignition in a Grote-Krekeler apparatus. However, the results were unsatisfactory as low values were obtained.

Results corresponding to the theoretical values were obtained in a Berthelot-Mahler-Klöcker bomb following the SCHULEK—CLAUDER method (128). The sulphuric acid evolved in the bomb was determined as barium sulphate.

Copper was determined by two different methods:

a) Gravimetrically, according to ERDEY (36). The sulphur-containing material was roasted in a porcelain pot, then, after dropwise addition of nitric acid, heated to constant weight. The residue was weighed as copper(II) oxide.

b) By means of the complexometric method of FLASCHKA (41). The pyridinocopper(II) thiobarbiturate complex was ignited in a platinum pot and the residue dissolved in dilute sulphuric acid with gentle warming. The product was titrated with 0.01 M complexon III in the presence of 6—8 drops of ammonia solution and a little murexide indicator. This procedure was also successful.

The results can be found in Table 5. Data listed in that Table are average values of the results of at least 3 parallel determinations within 0.1% deviation.

The melting point of each complex can be well determined. Before melting the material becomes brownish black, i. e. it melts with decomposition. In the case of $Cu(II)(py)_2(EMPTB)_2$, $Cu(II)(py)_2(EMBTB)_2$ and $Cu(II)(py)_2(AMPTB)_2$ the melting points are higher than that of the original thiobarbituric acid while the melting point of the ACHTB complex showed a 20° depression.

The above data prove that the starting material is suitable for analytical investigations, and that the composition of the pyridinocopper(II) thiobarbiturate complexes corresponds to the composition of:

$$(TB)_2Cu(py)_2.$$

ZWIKKER's formula (159, 160) for the pyridinocopper(II) complex of diethylbarbituric acid is

$$(barb)_2Cu(py)_2$$

essentially in agreement with our results.

Table 5
Investigation of the composition of pyridinocopper(II) thiobarbiturate complexes

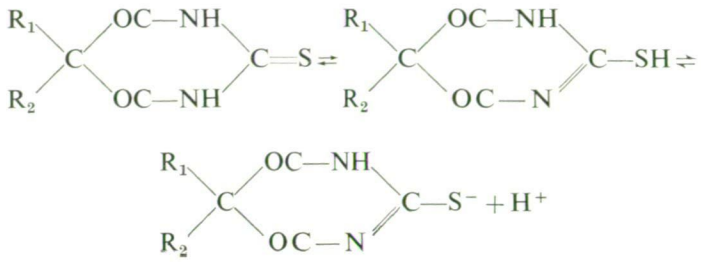
Complex	Mol. weight	C %		H %		N %		S %		Cu %			M. p. °C
		Calcu-lated	Found	Calcu-lated	Found	Calcu-lated	Found	Calcu-lated	Found	Calcu-lated	Gravi-metri-cally	Com-plexo-metrically	
Cu(II) $(py)_2$ $(EMPTB)_2$	676,36	53,28	53,65	5,96	6,01	12,42	12,14	9,48	9,73	9,39	9,52	9,40	132
Cu(II) $(py)_2$ $(EMBTB)_2$	704,41	54,56	54,76	6,30	6,49	11,93	12,33	9,48	9,02	9,39	9,25	9,33	126
Cu(II) $(py)_2$ $(AMPTB)_2$	700,38	54,88	55,10	5,76	5,90	11,99	11,85	9,16	9,49	9,07	8,99	8,80	127
Cu(II) $(py)_2$ $(ACHTB)_2$	748,42	57,77	57,52	5,38	5,22	11,22	11,08	8,55	8,34	8,49	8,31	8,30	159

Thus, similarly to that with the barbiturates, the complex formation with thiobarbiturates takes place according to the following scheme:

I. Reaction of copper sulphate with pyridine:

$$Cu^{2+} + 4\,py \rightarrow [Cu(py)_4]^{2+}$$

II. In aqueous pyridine the thiobarbiturates partially enolize and ionise:



III. Reaction of a thiobarbituric acid derivative with pyridinocopper(II):

$$[Cu(py)_4]^{2+} + 2TB \rightarrow [Cu(py)_2(TB)_2]^{2+} + 2py$$

Consequently, it is the above formula that corresponds to the composition of pyridinocopper(II) thiobarbiturate complexes, on the basis of the analogy with the pyridinocopper(II) barbiturate complexes, the analytical data, and some considerations of coordination chemistry.

The stabilities of pyridinocopper(II) thiobarbiturate complexes were examined in a drying oven at different temperatures during equal periods of time. The complexes are quite stable for 60 minutes at 70 °C: neither loss of weight nor colour change occurs. At 105 °C for 60 minutes the EMPTB and AMPTB complexes suffer a slight loss of weight. In the case of the EMBTB and ACHTB complexes the loss of weight is greater. The colour of the complexes turns from a dark shade to a lighter one. Hence, before analysis, they were dried over concentrated sulphuric acid and phosphorus pentoxide. The observed changes are summarized in Table 6.

Table 6

Investigation of the stability of pyridinocopper(II) thiobarbiturate complexes during drying

$Cu(II)(py)_2(TB)_2$ complex	Heating at 105 °C for 60 minutes	
	loss in weight	colour change
EMPTB	1,3%	+
AMPTB	1,8%	+ +
EMBTB	10,3%	+ + +
ACHTB	10,8%	+ + +

Note: + slight
+ + moderate
+ + + stronger

2.5. Derivatographic thermoanalysis of pyridinocopper(II) thiobarbiturate mixed complexes

During the determination of the composition of pyridinocopper(II) thiobarbiturate mixed complexes it was observed (95) that at temperatures above 105 °C the decomposition of these complexes occurs, accompanied by a colour change (see Chapter 2.4).

For a more detailed study of this phenomenon the complexes were analyzed by means of a derivatograph. In this way several useful data were obtained concerning the nature of the bonds in the complexes.

The pyridinocopper(II) complexes of EMPTB, EMBTB, AMPTB and ACHTB (most frequently used in therapy) were examined.

The data were recorded by means of a MOM derivatograph (PAULIK—PAULIK—ERDEY system) (109) using samples of 50—100 mg in a 9.5×12 mm platinum vessel, applying a weak suction; the heating rate was 12°/minute.

The derivatograms obtained can be seen in Figures 17—20.

The results of the calculations of the temperature values of the TG steps, based on analysis of the DTG and DTA curves, are shown in Tables 7 and 8.

Table 7

Analysis of pyridinocopper(II) thiobarbiturate mixed complexes on the basis of the TG curve

No.	Compound	Pyridine content %		2TB—2SH content %		residual CuO %	
		Calcd.	Found	Calcd.	Found	Calcd.	Found
1.	$Cu(II)(py)_2(EMPTB)_2$	25,4	up to 235°C 25,0	55,75	up to 350°C 58,7	11,76	12,5
2.	$Cu(II)(py)_2(EMBTB)_2$	22,49	up to 225°C 22,7	59,42	up to 350°C 60,0	10,29	10,9
3.	$Cu(II)(py)_2(AMPTB)_2$	22,59	up to 198°C 22,5	59,18	up to 380°C 60,0	11,35	12,0

Note: 2TB—2SH = the corresponding thiobarbiturate without an SH group, from which CuS is formed.

Table 8

Analysis of pyridinocopper (II) thiobarbiturate mixed complexes on the basis of the TG curve

Compound	2—pyridine + 1—cyclohexenyl + 1—ACHTB content (without S)		1ACHTB + 1cyclohexenyl + 1 S content		Residual CuO %	
	Calcd.	Found	Calcd.	Found	Calcd.	Found
$Cu(II)(py)_2(ACHTB)_2$	62,78	up to 465°C 62,8	20,55	up to 560°C 20,0	10,62	11,4

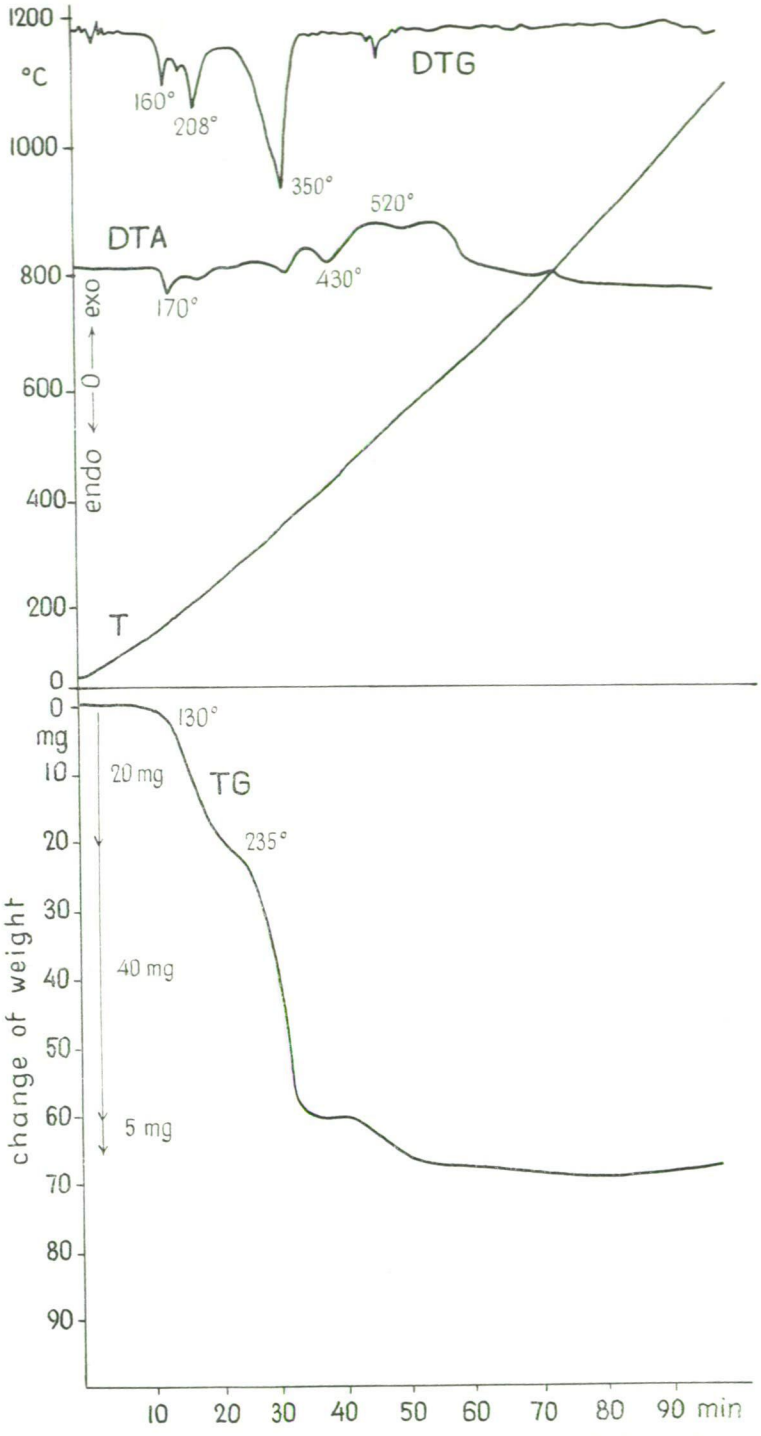


Fig. 17: Derivatogram of the Cu(II)$(py)_2(EMPTB)_2$ mixed complex.

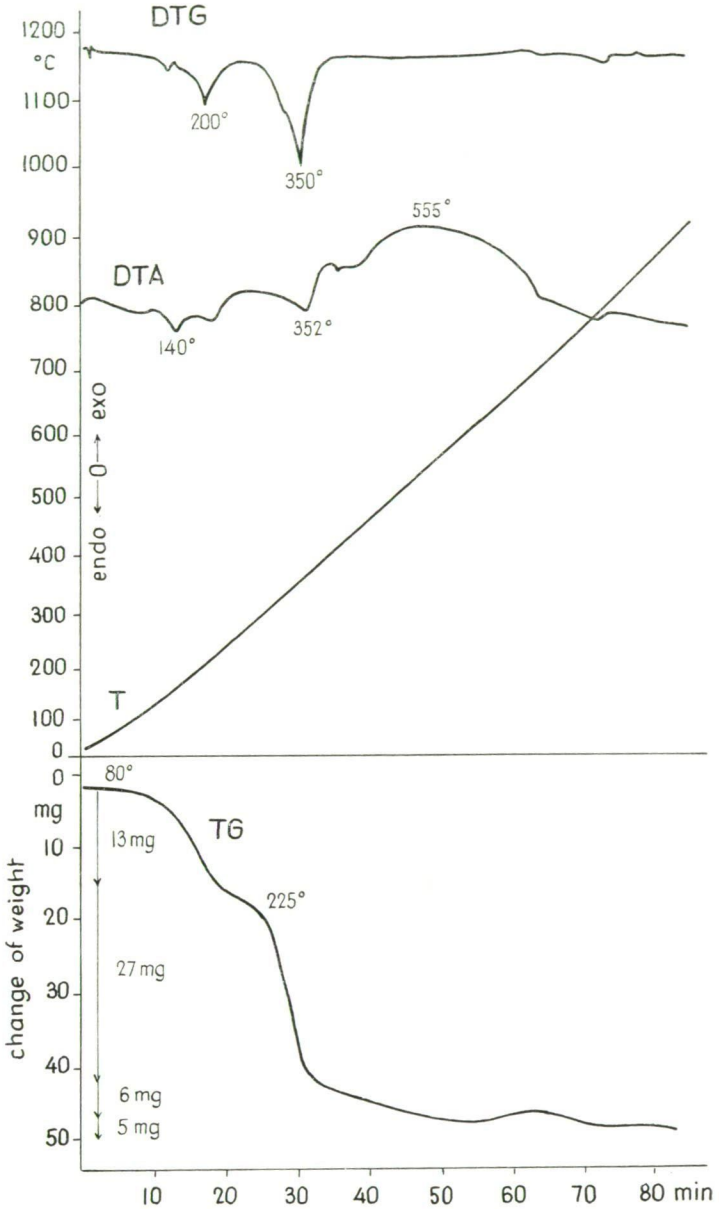


Fig. 18: Derivatogram of the $Cu(II)(py)_2(EMBTB)_2$ mixed complex.

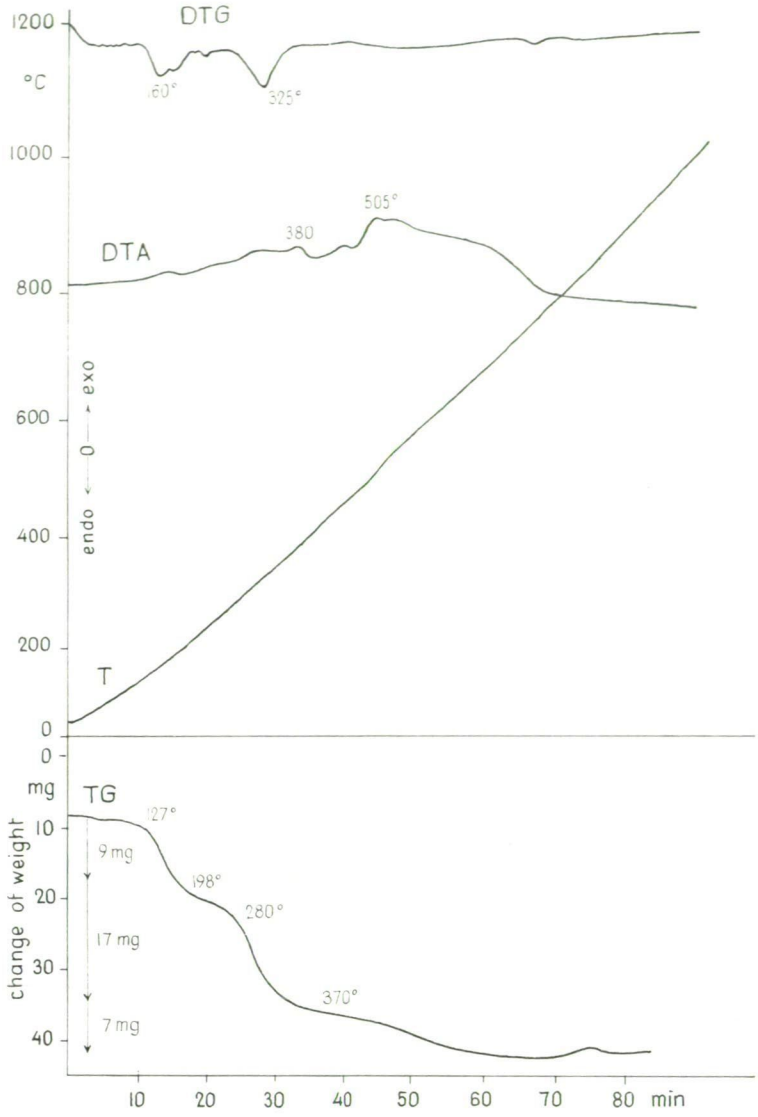


Fig. 19: Derivatogram of the $Cu(II)(py)_2(AMPTB)_2$ mixed complex.

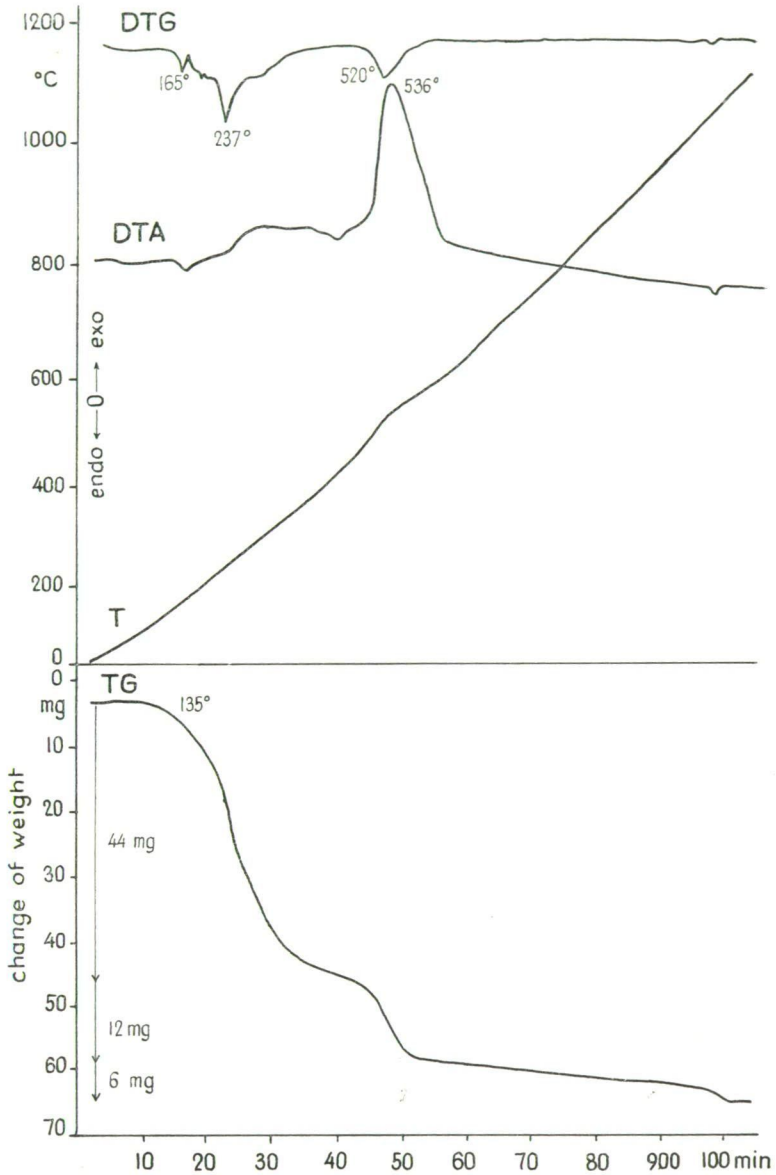


Fig. 20: Derivatogram of the Cu(II)$(py)_2(ACHTB)_2$ mixed complex.

The chemical and physical regularities of the thermal decomposition of heavy metal complexes containing organic molecules are little known. KŐRÖS et al. (71) carried out the thermoanalisys of some cobalt(II)-pyrazine mixed complexes. Barbituric acid and barbital were investigated thermogravimetrically by BERLIN and co-workers (9). The only data to be found concerning the thiobarbiturates are those of RISTICI and his associates (117, 118) who determined the water content of cadmium thiobarbiturate. The bonding energy of organic compounds and ligands measured at room temperature changes on heating; changes can also be brought about by interactions between neighbouring groups; it is also possible for the decomposition products to react with the parent compound or with each other, so that the thermal change may take a new direction. This applies especially to the heavy metal mixed complexes which are rather complicated systems, and is probably the reason why such problems have hardly been investigated quantitatively so far (108). However, it appeared from our investigations that thermoanalysis of the pyridinocopper(II) thiobarbiturate mixed complexes gave TG steps and DTG, DTA curves which could be well evaluated. At the same time these data supported our examinations concerning the composition of the complexes (95) and permitted conclusions to a certain extent as to their structures which were proved by IR spectral analysis (104).

Thermoanalytical investigations of the pyridinocopper (II) EMPTB, EMBTB and AMPTB mixed complexes clearly showed that in the course of the thermal decomposition the pyridine (b. p. 115.3 °C) was first removed from the system between 125—235 °C, followed by the thiobarbituric acid derivative between 350—380 °C, having gained sufficient energy for break age of the C—S bond (67.0 Kcal/mole); the sulphur formed copper(II) sulphide which then vapourised (b. p. 445 °C).

Since the m. p.'s of the investigated thiobarbituric acid derivatives are between 139 and 160 °C, above this temperature one has to consider their thermal decomposition.

The m. p. of thiobarbituric acid is 233 °C. The energy of the C=S bond is 117.8 Kcal/mole, that of the C=N bond is approximately 145 Kcal/mole and that of the C—N bond is 68 Kcal/mole (35).

Our data suggest that in the complex the copper(II)-pyridine bond can be decomposed by smaller energy than the S—C bond. After the thiobarbiturate has left, the system, a residue of copper(II) sulphide remains.

The thermogravimetric and derivative thermogravimetric curves of copper(II) sulphide are known from the work of PAULIK and LIPTAY (107). The residue loses surface-adsorbed water at about 100 °C; then, above 130 °C, sulphur is lost at an increasing rate and the composition of the solid approximates to that of Cu_2S. However, before reaching the latter, at about 350 °C, oxidation commences and takes place in two steps. The maximum increase of weight corresponds to the transformation of about 12% of the CuS into copper(II) sulphate, and the TG maximum occurring at 640 °C to the composition CuO. $CuSO_4$. Up to 710 °C this compound decomposes quite slowly; the rate of decomposition is highest at 740 °C, while at 820 °C the precipitate is completely converted into copper(II) oxide. At 940 °C this also starts to decompose to copper(I) oxide, wich is obtained in a pure form only by keeping the material at 1050 °C.

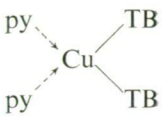
According to the TG curve only the horizontal section, i. e. between 820—940 °C corresponding to the composition of CuO, is suitable to obtain the solid into a form of constant composition.

The above picture can be recorded and measured on the mg scale only with

difficulty due to the small copper content of the complex. Thus, instead of the expected maxima of the curves, in several places only inflections can be found.

Decomposition of the two ACHTB complexes (containing cyclohexenyl groups) is different from those described above. Analyses showed that in the first step not only pyridine but also thiobarbituric acid and cyclohexene decomposition products are lost. This is to some extent understandable considering the lack of symmetry of the compound which may explain the different decomposition. CuS and CuO residues are also formed in this case.

Our earlier statements concerning the composition and structural formulae of complexes are supported by the derivatographic data. Accordingly, the general formula of these complexes is the following:



The copper is bonded to the thiobarbiturate through the sulphur atom (104).

2.6. UV spectra of the pyridinocopper(II) thiobarbiturate mixed complexes

The UV spectra of Cu(II)$(py)_2(EMPTB)_2$, Cu(II)$(py)_2(EMBTB)_2$, Cu(II)$(py)_2$ $(AMPTB)_2$, and Cu(II)$(py)_2(ACHTB)_2$ mixed complexes were taken with a Beckman DU spectrophotometer. The samples dissolved in methanol were measured in 0.1—1 cm quartz cells at room temperature against methanol. The spectra are shown in Figures 21 and 22.

As may be seen from Fig. 21, the spectra of Cu(II)$(py)_2(EMPTB)_2$ and Cu(II) $(py)_2(EMBTB)_2$ are completely similar. There is a characteristic sharp peak at 288 nm, and further maxima at 238 and 342 nm (the latter is broad and flattened).

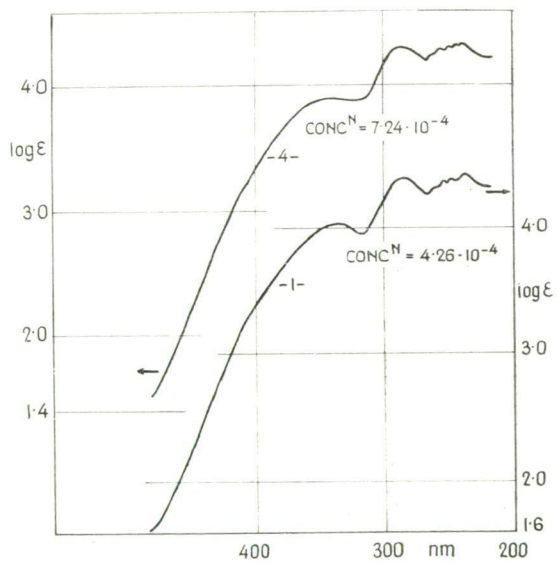


Fig. 21: Curve 1: Cu(II)$(py)_2(EMBTB)_2$ Curve 4: Cu(II)$(py)_2(EMPTB)_2$

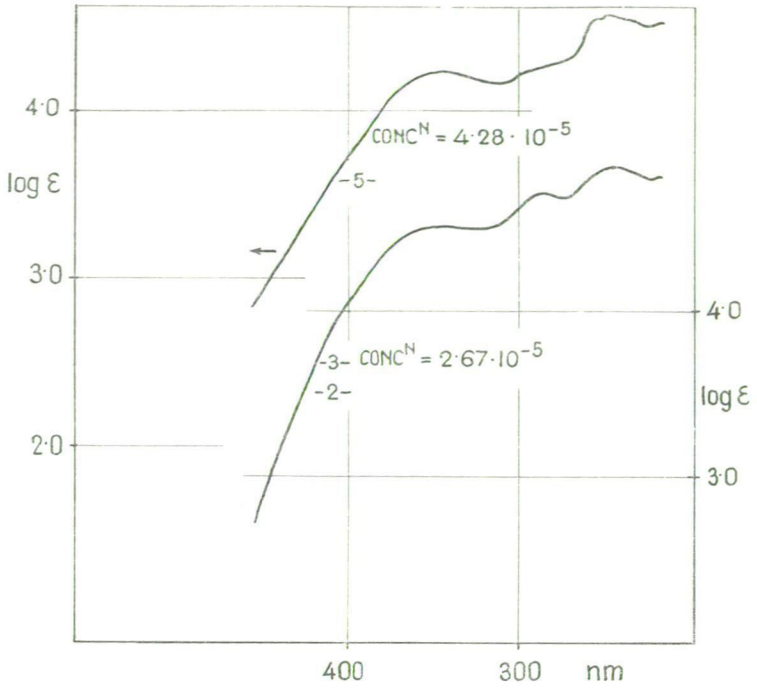


Fig. 22: Curves 2, 3: $Cu(II)(py)_2(ACHTB)_2$ Curve 5: $Cu(II)(py)_2(AMPTB)_2$

The curves of $Cu(II)(py)_2(AMPTB)_2$ and $Cu(II)(py)_2(ACHTB)_2$ (Fig. 22) are also similar. Broad maxima are found at 260 and 344 nm. $Cu(II)(py)_2(ACHTB)_2$ shows an additional maximum at 288 nm which appears on the spectrum of $Cu(II)(py)_2(AMPTB)_2$ as only a slight inflexion.

As a result of these results it seems that their UV spectra are not suitable to distinguish between complexes of closely related structure. Nevertheless, the UV spectra of a pyridinocopper(II) thiobarbiturate complex containing a 5-allyl group differs from that of the complex where the 5-allyl is replaced by a 5-ethyl group. The differences can be readily observed in the UV region of the curves.

2.7. Analysis of the absorption and reflectance spectra of copper(II) thiobarbiturate and pyridinocopper(II) thiobarbiturate complexes

Cu(II) ions and the thiobarbiturates form compounds of composition $Cu(TB)_2$ (100) and, in the presence of pyridine, a complex of $Cu(II)(py)_2(TB)_2$ (95). This Chapter deals with the spectroscopic investigation of the above compounds.

EMBTB, AMPTB, ACHTB and EMPTB were precipitated with hydrochloric acid from the corresponding sodium salts (used as intraveneous narcotics) and purified by recrystallization. The sodium salts served as starting materials for the preparation of the $Cu(TB)_2$ complexes as follows: 1.5 g of the sodium salt dissolved in 100 ml of methanol was added to a solution of 0.5 g of $CuSO_4$. 5 H_2O in 100 ml of methanol and the mixture allowed to stand for 24 hours. During this time the solution became green. It was concentrated in vacuo to 30 ml and the complex formed was precipitated with water. After filtration it was washed with methanol and water until free from sulphate, and dried in vacuo over sulphuric acid and finally phosphorus pentoxide.

The $Cu(II)(py)_2(TB)_2$ mixed complexes were prepared as described in Chapter 2.2.

Absorption and reflectance spectra were measured with a Beckman DU spectrophotometer at room temperature in the region 210—1250 nm. Suitably purified methanol was used as solvent. The reflectance spectra were measured against MgO standards without dilution. Characteristic spectral data are summarized in Table 9.

Some characteristic spectra are shown in Figures 24—26.

It is well known (5, 126) that the Cu^{2+} ion, of $[Ar]d^9$ $[(t_{2g})^6(e_g)^3]$ electronic configuration, can form octahedral, square planar and tetrahedral complexes; the two former structures being more frequent. In the spectrum of six-coordinated $[Cu(H_2O)_6]^{2+}$ only one wide complex band can be found, at $\sim$800 nm; this is significantly shifted ($\sim$680 nm) if the water molecules are replaced by NH_3. Owing to the JAHN—TELLER effect (57, 58) the symmetry of these molecules is decreased and the bands are always complex. The main band may be resolved into several (three in general) subsidiary bands (8).

In accordance with the stoichiometry of $Cu(II)(py)_2(TB)_2$ there are two structural possibilities: a low symmetry tetrahedron or a square plane. The ninefold degenerate ground state of the central ion is split in these cases as shown in Fig. 23. It is obvious that the splitting of the ground state differs considerably in the two geometric configurations. In the case of the square plane the d_z2 level may be of lowest energy, depending on the field strength of the ligands. It has been pointed out that the crystal field stabilization energy of the square plane is much higher than that of the

Table 9

UV, visible and near IR spectral data of compounds of type $Cu(TB)_2$ and $Cu(II)(py)_2(TB)_2$

Complex	Concentration x 10^3	Peak maxima (nm, log ε)							
$Cu(EMPTB)_2$	D. R.[a]	880		750	570	—	—	—	
	12,7	—	690*	(1,44)	610	338 (3,88)	288 (4,17)	236	(4,18)
$Cu(II)(py)_2$ $(EMPTB)_2$	D. R	740		645	522	—	—	—	
	7,24	—	690*	(1,52)	—	343 (3,87)	287 (4,28)	238	(4,30)
$Cu(EMBTB)_2$	D. R.	870		730	600	—	—	—	
	6,17	—	705*	(1,56)	—	345 (3,95)	286 (4,14)	236	(4,20)
$Cu(II)(py)_2$ $(EMBTB)_2$	D. R.	850		700	550	—	—	—	
	4,26	—	695*	(1,76)	—	341 (4,00)	288 (4,42)	239	(4,43)
$Cu(AMPTB)_2$	D. R.	870		745	—	—	—	—	
	4,7	—	700*	(1,79)	—	346 (4,01)	284 (4,21)	236	(4,28)
$Cu(II)(py)_2$ $(AMPTB)_2$	D. R.	800		645	538	—	—	—	
	4,28	—	690*	(2,20)	—	345 (4,22)	290 (4,23)	248	(4,54)
$Cu(ACHTB)_2$	D. R.	860		760	—	—	—	—	
	6,49	—	715*	(1,38)	—	345 (3,86)	288 (4,27)	236	(4,16)
$Cu(II)(py)_2$ $(ACHTB)_2$	D. R.	—		655	530	—	—	—	
	2,67	—	680*	(1,98)	—	342 (4,30)	287 (4,70)	246	(4,84)

a) D. R. = diffuse reflectance spectrum
* = complex, asymmetric bands

tetrahedral structure. The latter is formed only when the planar arrangement is prevented by steric hindrance. In the case of a square plane the ground state is not degenerate; accordingly, there is merely spin-only momentum and no angular momentum contribution. The measured value is about 1.73 BM.

From the available data it may be concluded that the complexes in question have square planar configurations. The electronic configuration of the Cu^{2+} ion is d_ε^6, d_{xy}^2, $d_{x^2-y^2}$, and the ligand bonding electrons form a dsp^2 hybrid. This naturally requires excitation of an electron probably to the 4d level. So the electronic configuration of the complex molecule is:

$$[Ar]d_\varepsilon^6,\ d_{xy}^2,\ d_{x^2-y^2}^2,\ 4s^2,\ 4p_x^2,\ 4p_y^2,\ 4d^1.$$

In the case of a strong field the bonding of the ligands in the $d_{x^2-y^2}$ direction is so strong and they are so near to the metal ion that they repel the d_{xy} electrons whereby their energy decreases.

In accordance with the foregoing, the centres of the principal bands in the spectra of the compounds investigated are found between 640 and 760 nm. However, the shape and structure of the solution and reflection spectra differ essentially. The complexity of the bands may be observed in the case of solid $Cu(TB)_2$ compounds,

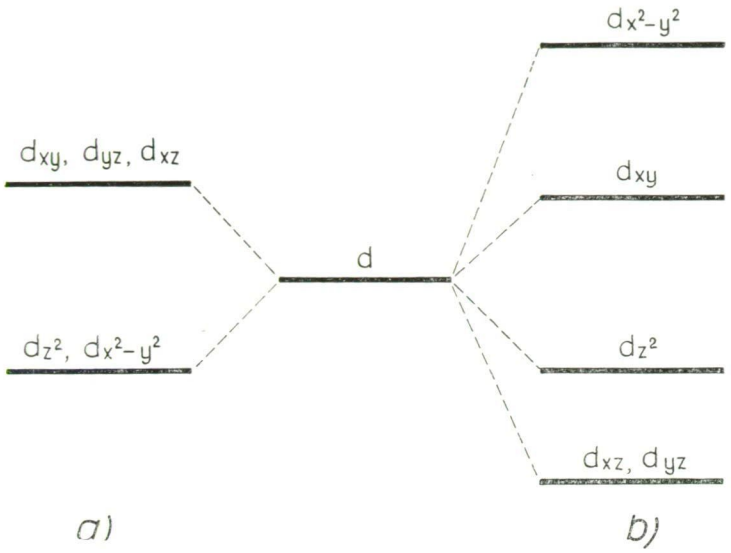


Fig. 23: Schematic splitting of the ground state of the Cu^{2+} ion in a) a tetrahedral, and b) a square planar field.

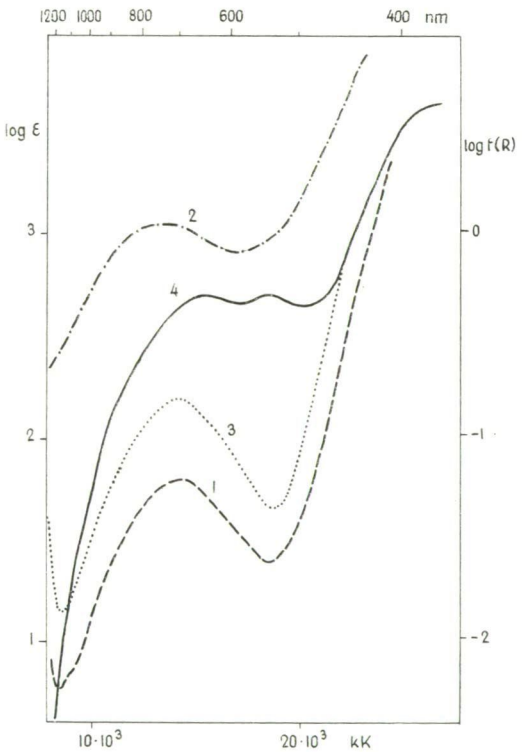


Fig. 24: Visible absorption and reflectance spectra. 1: Cu(II)(AMPTB)$_2$ 2: Cu(II)(AMPTB)$_2$, D. R. 3: Cu(II)(py)$_2$(AMPTB)$_2$ 4: Cu(II)(py)$_2$(AMPTB)$_2$, D. R. (1,3 in methanol)

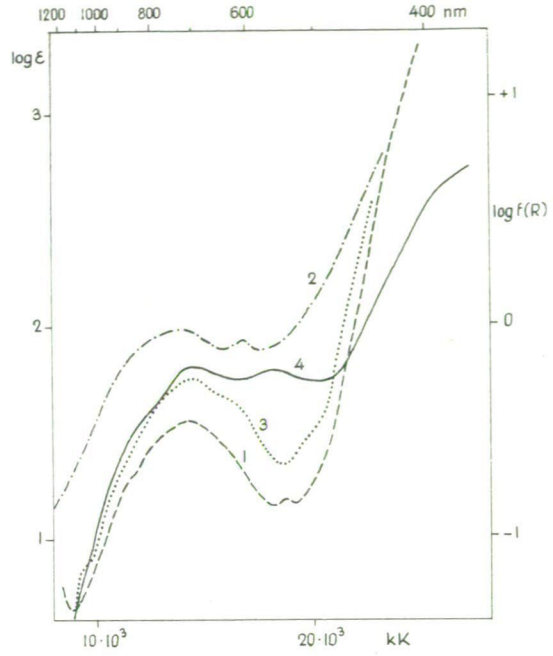


Fig. 25: Visible spectra. 1: $Cu(EMBTB)_2$ in methanol; 2: $Cu(EMBTB)_2$, D. R.; 3: $Cu(II)(py)_2(EMBTB)_2$ in methanol; 4: $Cu(II)(py)_2(EMBTB)_2$, D. R.

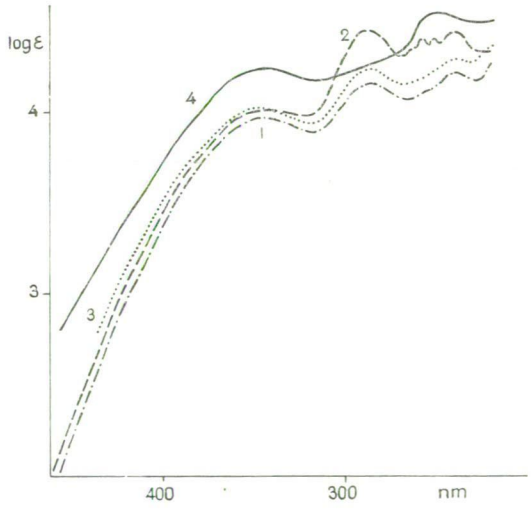


Fig. 26: UV absorption spectra in methanol. 1: $Cu(EMBTB)_2$; 2: $Cu(II)(py)_2(EMBTB)_2$; 3: $Cu(AMPTB)_2$; 4: $Cu(II)(py)_2(AMPTB)_2$.

and even more clearly with the $Cu(py)_2(TB)_2$ complexes. Applying the method of curve analysis it is not difficult to demonstrate at least three subsidiary bands in agreement with the energy-level splitting (Fig. 23). It can be concluded that in the case of $Cu(TB)_2$ the centres of the principal bands change in the order

$$EMBTB < AMPTB < EMPTB < ACHTB$$

and with $Cu(II)(py)_2(TB)_2$ compounds in the following way:

$$AMPTB = EMPTB < ACHTB < EMBTB$$

Moreover, in all cases

$$\lambda Cu(TB)_2 > \lambda Cu(II)(py)_2(TB)_2,$$

that is, the bonding character of the pyridine-containing compounds shifts significantly in the covalent direction. The ligands examined can be put in the spectrochemical series between H_2O and NH_3.

In solution the complexity of bands decreases to some extent. In the case of AMPTB and ACHTB the band is nearly symmetric. The UV spectra of the solutions are similar for each complex. Three well-defined bands can be measured for $Cu(TB)_2$ compounds at about 340, 286, and 236 nm, and for compounds of type $Cu(II)(py)_2(TB)_2$ at about 343, 288, and 242 nm. Their intensity is generally $\varepsilon \geqq 10{,}000$. Considering the UV spectra of the free thiobarbiturates, the two bands of shorter wavelength can be assigned to the excitation of the electronic system of the ligands ($\pi \rightarrow \pi^*$ transition). These bands also are complex because the $\sim$245 nm band involves the characteristic $\sim$250 nm band of free pyridine as well. The typical vibration structure of pyridine can be well observed in the case of EMBTB and EMPTB (Fig. 26). The band at about $\sim$340 nm can be regarded as a charge transfer band between the central ion and the ligand ($d_{\gamma} - \pi^*$ transition). An atomic orbital of the Cu and an empty π^* excited orbital of the pyridine (or thiobarbiturate) are involved in this process (62, 156). It is remarkable that these charge transfer bands appear at essentially higher energy than in the simple CuX_2py_2 ($X = Cl^-$, Br^-) compounds (62, 156).

2.8. Analysis of the absorption and reflectance spectra of cobalt(II) thiobarbiturate complexes

The PARRI reaction (106), based upon the complex formation between barbituric acid derivatives and the cobalt(II) ion, has been applied by several authors (53, 99) for the determination of thiobarbiturates.

Data concerning the structure of barbiturate complexes were reported by ZWIKKER (160). He found a 1:2 cobalt(II):barbiturate ratio.

For the partial elucidation of the structure of cobalt(II) thiobarbiturate complexes we have analysed the absorption and reflectance spectra of these complexes on the basis of the ligand field theory and refer to their magnetic properties. Results of the latter measurements can give valuable information concerning structural and bonding conditions of the complexes (39).

In our experiments the cobalt(II) complexes of EMBTB, EMPTB, AMPTB and ACHTB were examined. These complexes were prepared in crystalline form as follows.

100 ml of a 1.5% methanolic solution of the sodium salt of the relevant thiobarbituric acid was added to 100 ml of a 0.5% methanolic solution of $CoCl_2 . 6 H_2O$, and the mixture allowed to stand for 24 hours. During this time the solution became green. Its volume was reduced in vacuo to 30 ml and the complex formed precipitated by addition of water. The product was filtered, washed with methanolic water, and dried in vacuo over sulphuric acid and finally phosphorus pentoxide.

In the case of EMPTB the solution was violet-coloured until it was made alkaline with sodium hydroxide. Afterwards it was treated as described above.

Absorption and reflectance spectra were measured with a Beckman DU spectrophotometer at room temperature in the region from 210 to 1200 nm. Readings were taken between 1200—800 nm at 5 nm intervals, between 800—400 nm at 4 nm intervals, from 400 to 300 nm at 2 nm intervals, and at shorter wavelengths at 1 nm intervals. Methanol was used as solvent, and p. a. MgO as the reflectance standard. Reflectance spectra were measured without dilution of the samples, and for calculations the KUBELKA—MUNK relation (73, 74) was applied. Spectra are shown in Figs. 27—29 and their characteristic data are summarized in Table 10.

From a survey of the Figures it is evident that the spectral structures of all the compounds are similar to a great extent. In the UV spectra of methanolic solutions (Fig. 27) characteristic maxima can be found at about ~238 and 280—290 nm. The structure in this spectral region is practically independent of the substituents in position 5 as has already been stated by POETHKE and PREUSSLER (114) in connection with the free thiobrabiturates. Only in the case of EMPTB do the characteristic

Table 10

Measured and calculated spectral data of compounds of type CoR_2X_2

R =	Concentration x 10^3 M	Intraligand bands		d-d bands		f ν^*_3 x 10^4
		ν^*I	ν^*II	ν^*2	ν^*3	
EMPTB	4,26	255 (4,26)	306 (4,41)	1140 (0,58) 944 (0,59)	615 (2,12)	5,8
	refl.	—	—	1070	621 584	—
EMBTB	5,32	237 (4,28)	287 (4,52)	1060 (0,58)	615 (1,82)	4,4
	refl.	—	—	1075	615	—
AMPTB	3,48	239 (4,25)	265 (4,25)	1025 (1,00)	611 (2,23)	11,3
	refl.	—	—	1110	615	—
ACHTB	4,2	238 (4,37)	285 (4,51)	1040 (0,64)	605 (2,00)	7,4
	refl.	—	—	1100	600	—

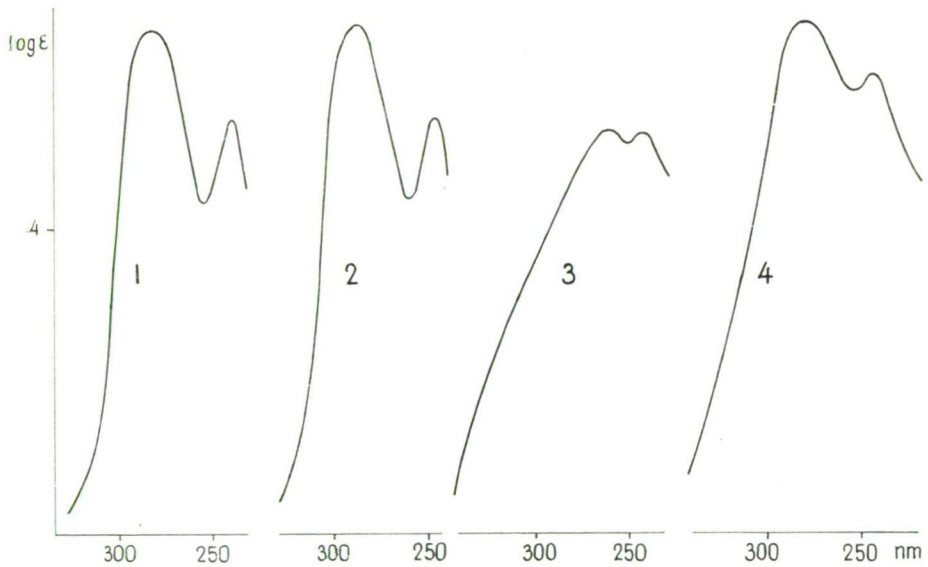


Fig. 27: UV absorption spectra of compounds of type CoR_2X_2 in methanol. R = 1: EMBTB; 2: EMPTB; 3: AMPTB; 4: ACHTB

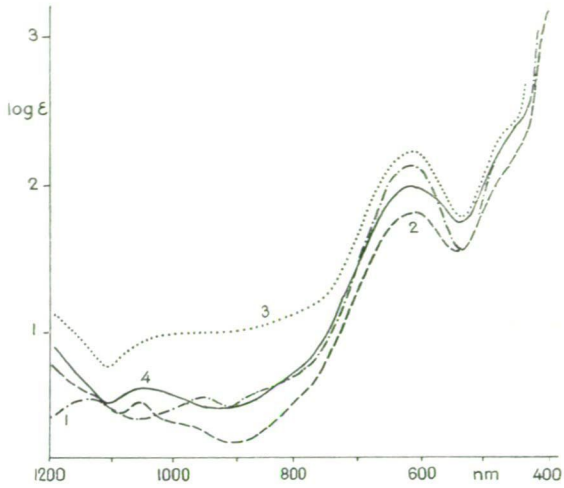


Fig. 28: Absorption spectra of methanolic solutions of compounds of type CoR_2X_2. 1: EMPTB; 2: EMBTB; 3: AMPTB; 4: ACHTB

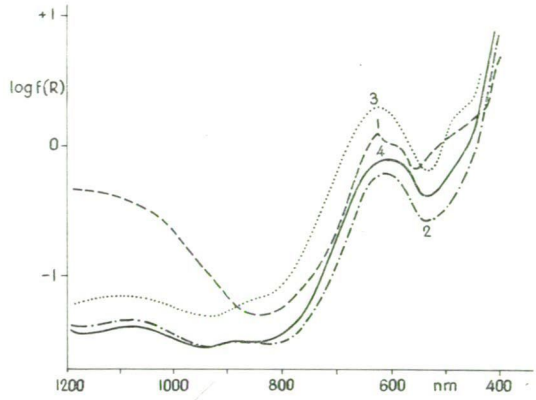


Fig. 29: Reflectance spectra of compounds of type CoR_2X_2. R = 1: EMPTB; 2: EMBTB; 3: AMPTB; 4: ACHTB

bands shift (to 255 and 305 nm) but, nevertheless, the structure of the curve is unchanged.

Characteristic absorption in this region can be produced only by excitement of the electronic system ($\pi \rightarrow \pi^*$ transition) of the *N* or *S* atoms. However, the possibility of different charge transfer processes (metal ion $\rightleftharpoons$ ligand) must be considered, to other atom groups do not absorb significantly in this region. The nearly identical transition energy values indicate that neither basicity difference of the ligands nor C_5 substituents essentially influence the excitation energies. In fact, the UV region gives the basic spectral structure of the ligands as has already been observed in the case of other complexes containing ligand(s) of characteristic absorption (80). Calculations concerning the oscillator strength also refer to intraligand transitions.

Spectral structure in the region above 340 nm is related to the electron system of the central metal ion and is characteristic. On the basis of the colour of the compounds, the structure of the ligands and the spectra, as well as the effect of the solvent on the absorption spectrum, a tetrahedral Co^{2+} compound is probable, with 4s, $4p_x 4p_y 4p_z$ hybrid bonds.

The seven-fold degenerate ^4F ground-state of the central metal ion with $(Ar)d^7$ electronic configuration ($S=3/2$) splits into two three-fold and one simple degenerate states (T_1, T_2, and A_2, resp.) in a potential field of tetrahedral symmetry.

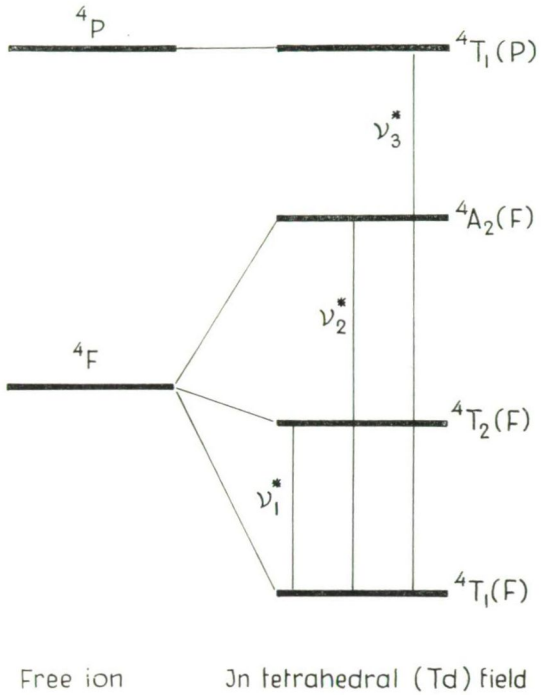


Fig. 30: Splitting of the ground state of the Co^{2+} ion in a potential field of tetrahedral symmetry, and the more important transitions.

Taking into consideration the different donor atoms, the molecules have CoS_2X_2 microsymmetry and belong to the C_{2v} point group (X is presumably OH^-). Considering also the interaction with the higher energy and identical multiplicity ^4P term, we expect three spin-allowed, LAPORTE-forbidden transitions or bands (5, 23, 126):

$$\nu_1^*: \ ^4T_2(F) \leftarrow {}^4A_2(F) = \Delta$$

$$\nu_2^*: \ ^4T_1(F) \leftarrow \quad = 1.5\Delta + 7.5B' - Q,$$

$$\nu_3^*: \ ^4T_1(P) \leftarrow \quad = 1.5\Delta + 7.5B' + Q,$$

where

$$4Q^2 = \Delta^2 - 18B'\Delta + 225B'^2, \quad Q = (\nu_3^* - \nu_2^*)3$$

and $$B' = \frac{\nu_2^* + \nu_3^* - 3\Delta}{15}.$$

With tetrahedral compounds the three bands appear between 3300—2000, 1300—1000, and 700—550 nm, respectively. Their intensities are generally as follows: $\varepsilon_{\nu 1}^* \leqq \varepsilon_{\nu 2}^* \ll \varepsilon_{\nu 3}^*$. The tetrahedral form is also made possible by the fact that the Co^{2+} ion, as a result of the value of its crystal field stabilization energy, forms tetrahedral complexes of high spin number much more easily than do the other transition metal ions (74).

The absorption and reflectance spectra of the complexes (Figs. 28, 29) both contain the ν_2^* and ν_3^* bands at about $\sim$1050 and $\sim$610 nm. The structures of the solution and reflectance spectra are identical i. e. no essential change occurs in the structure of the molecule on dissolution. The ν_3^* band is generally complex and broad; this indicates the presence of an additional quartet-quartet component. This is to be expected, considering the dissimilarity of the ligands coordinated to the Co^{2+} ion (or, rather, the donor atoms) and is traceable primarily to the splitting of the $^4T_1$ level brought about by the spin-orbit interaction. The band complexity is similar to that observed with the $[Co(py)_2X_2]$ ($X = Cl^-$, Br^-, I^-) compounds (37).

The tetrahedral complexes have no centre of symmetry, and thus the corresponding transitions are considerably more intense than with the octahedral compounds. For example, in the case of $(CoCl_4)^{2-}$ the intensity is about one hundred times that observed with $[Co(H_2O)_6]^{2+}$. The oscillator strength values for the ν_3^* band, calculated by means of the correlation $f = 4.6 \times 10^{-9}\ \varepsilon_{max}\delta\pm$, are of the order of 10^{-3}—10^{-4} (ε_{max} is the extinction of the maximum, $\delta\pm$ is the half-width of the band at $\varepsilon_{max}/^2$), this corresponds to the values observed (63) with tetrahedral complexes of the first row of transition metal ions (see Table 10).

According to the exploratory calculations based on the TANABE—SUGANO energy matrices (140), the ν_1^* band ($=\delta$) can be expected at about 2000 nm. With all the compounds, very close RACAH-B values (30), and consequently similar β nephelauxetic ($\beta = B'/B_0$, $B_0 = 971\ cm^{-1}$) values ($\sim$0.60), are obtained; this is proof that the bonds formed in the complex molecules are to a large extent of a covalent character.

Our magnetic measurements showed that the compounds are paramagnetic, with values of about $\sim$4.3 BM. With the use of this value, and with $\Delta \sim 5000\ cm^{-1}$, a constant spin-orbit coupling value of $\lambda^* \sim 145\ cm^{-1}$ is obtained; this is a significant decrease compared with $\lambda_0 = 178\ cm^{-1}$ calculated for Co^{2+} and indicates overlapping of the electron orbitals, i. e. considerable covalent character of the bonds formed.

2.9. Infrared spectral investigation of the structure of pyridinocopper(II) thiobarbiturate mixed complexes

Determination of the composition of pyridinocopper(II) thiobarbiturate complexes prepared according to the method described in Chapter 2.2. (95) unambiguously proved that their composition was corresponding to the $Cu(II)—(py)_2—(TB)_2$ general formula.

Since for the unambiguous determination of the structure of these complexes it is unsatisfactory to know their composition, this problem was studied in more detail.

There are no data in the literature concerning the structure of the thiobarbituratopyridinocopper(II) complexes. FIELKOV and RAPAPORT (38) suggested another structural formula for barbituratocopper(II) complexes in addition to ZWIKKER's (159) formula; considering a coordination number of six for copper.
This formula however, was not proved.

Several authors, for instance AWE and WINKLER (2), TSUKAMOTO and YOSHIMURA (145), HEISE and KIMBEL (53), and BAGGES GAARD—RASMUSSEN et al. (4) suggested that for the ZWIKKER (159) reaction to take place it is necessary that the

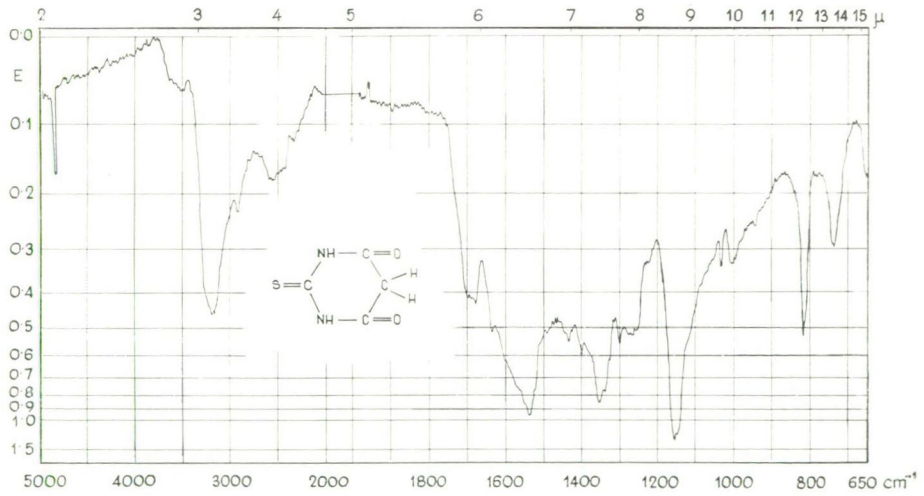


Fig. 31: IR spectrum of 2-thiobarbituric acid.

groupings $=N-\underset{\underset{O}{\|}}{C}-N=$ and/or $-N=C-N=$ be present. However, it has been pointed out by MOHRSCHULZ (90) that some substances not containing these groupings (e. g. sulphonamides) give positive reactions. Finally, UMBERGER and ADAMS (146) and LEVI and HUBLEY (75) proved the structure of barbituratopyridinocopper(II) by means of IR spectral data.

In order to determine the structure of the thiobarbituratopyridinocopper(II) complexes, we prepared thiobarbituric acid, some of its 5,5-substituted derivatives, and the corresponding complexes, as already described (95), and their IR spectra were taken.

Spectra were recorded with a Unicam SP 200 spectrometer. Samples were prepared using Merck Uvasol KBr. The IR spectra recorded are shown in the following Figures.

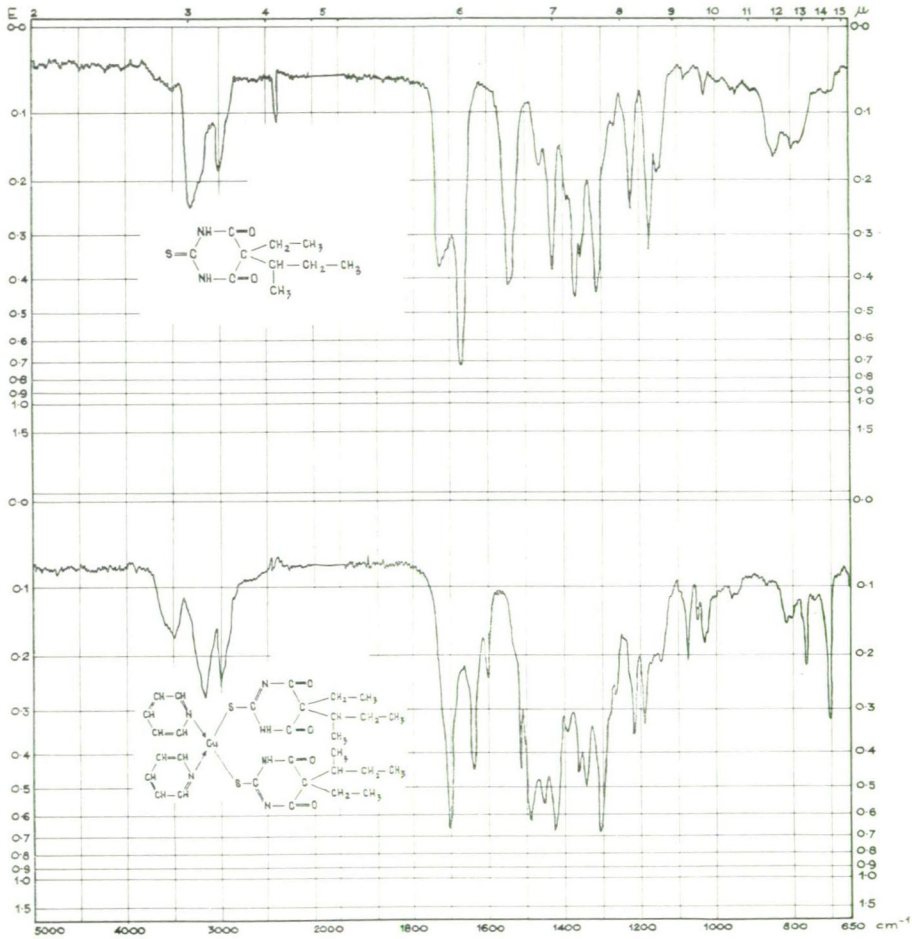


Fig. 32: IR spectra of EMPTB and Cu(II)—$(py)_2$—$(EMPTB)_2$ complexes.

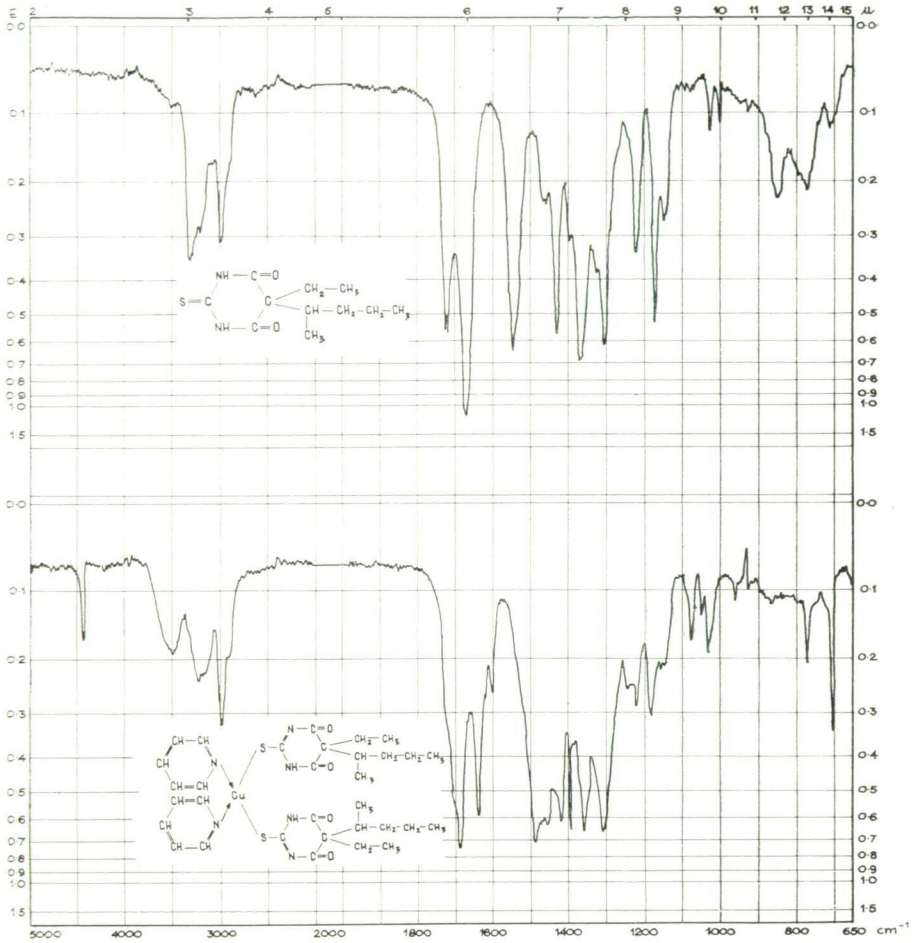


Fig. 33: IR spectra of EMBTB and Cu(II)—$(py)_2$—$(EMBTB)_2$ complexes.

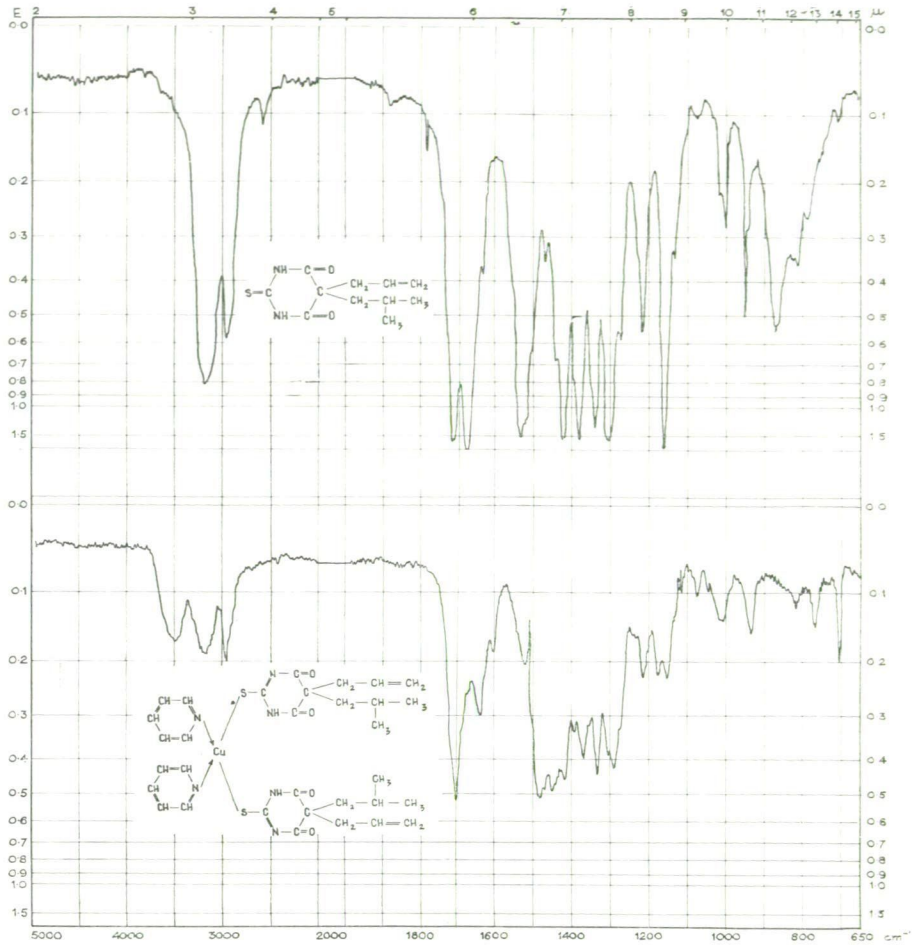


Fig. 34: IR spectra of AMPTB and Cu(II)—$(py)_2$—$(AMPTB)_2$ complexes.

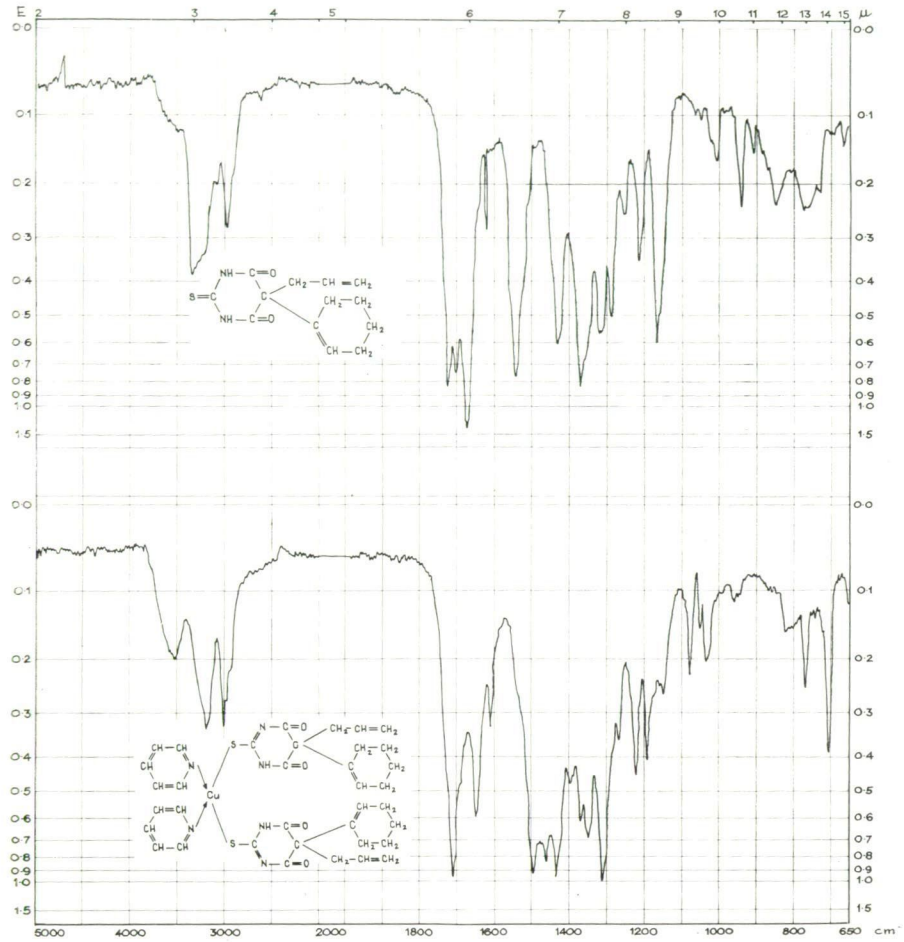


Fig. 35: IR spectra of ACHTB and Cu(II)—$(py)_2$—$(ACHTB)_2$ complexes.

The 5,5-substituted thiobarbituric acids show intense N—H stretching absorption in the region 3400—3200 cm^{-1}. In this case, the absorption, in contrast to that of the barbituric acid derivatives, is not a doublet. Thus, in the case of thiobarbituric acid, the absorption between 3400—2200 cm^{-1} is presumably not only due to the NH stretching vibrations.

This is supported by the fact that thiobarbituric acid gives a comparatively weak doublet in the carbonyl absorption region at 1680 and 1700 cm^{-1}, while the doublets due to the 5,5-substituted thiobarbituric acid derivatives are much more intense and appear at 1670 and 1720 cm^{-1}. The band appearing at about 1720 cm^{-1} in the case of 5-allyl-5-cyclohexenylthiobarbituric acid was split into a doublet. The absorption in the region 1670 cm^{-1} is in all cases much more intense and due to the other $\nu C=O$ stretching.

The Amide III band of the compounds investigated appears, as in other thioamides, at 1150—1180 cm^{-1} in all cases, but has the highest intensity in the case of thiobarbituric acid. The allyl-substituted thiobarbituric acids show γCH and $\nu C=C$ absorptions due to the allyl group at 960, 1010, and 1635 cm^{-1}.

Many absorptions characteristic of pyridine complexes are found in the case of thiobarbituratopyridinocopper(II) complexes (162). From among the ring-vibrations the ν ring(A_1) is clearly observable at 1608 cm^{-1} in contrast to the corresponding barbituric acid complexes (LEVI—HUBLEY; 75), in which it is concealed by the strong (Amide I) band. The out-of-plane deformations $\gamma CH(B_2)$ were also observed at about 705 cm^{-1}.

In all cases the complexes failed to give any thioamide stretching absorption, indicating that the copper atom is bound to the sulphur atom. The Cu—S band could not be detected, probably due to its position and low intensity (48).

In the complexes both the $\nu N—H$ and $\nu C=O$ stretching bands showed characteristic differences compared with related bands of thiobarbituric acids. The $\nu N—H$ stretching band appears as a doublet with maxima at 3200 and 3500 cm^{-1}.

Table 11

IR absorption maxima of thiobarbituric acid derivatives

	N—H (cm^{-1})	C=O (cm^{-1})	C=S (cm^{-1})	allyl (cm^{-1})
thiobarbituric acid	3200	1700, 1680	1158	—
5-allyl-4-(2-methylpropyl) thiobarbituric acid AMPTB	3233	1718, 1678	1165	1637, 1008 957
5-ethyl-5-(1-methylbutyl) thiobarbituric acid EMBTB	3350, 3200	1726, 1670	1172	—
5-ethyl-5-(1-methylpropyl) thiobarbituric acid EMPTB	3200	1724, 1672	1175	—
5-allyl-5-(1-cyclohexenyl) thiobarbituric acid ACHTB	3200	1718, 1674 1702,	1170	1628, 1008, 942

Table 12

IR absorption bands characteristic of the pyridine in the pyridinocopper(II) thiobarbiturate complexes*

	Ring (A_1) (cm^{-1})	Ring (B_2) (cm^{-1})	CH (B_2) (cm^{-1})
5-allyl-5-(2-methylpropyl) thiobarbituric acid AMPTB	1603 w	770 w	708 m
5-ethyl-5-(1-methylbutyl) thiobarbituric acid EMBTB	1605 w	772 w	710 m
5-ethyl-5-(1-methylpropyl) thiobarbituric acid EMPTB	1605 w	768 w	708 m
5-allyl-5-(1-cyclohexenyl) thiobarbituric acid ACHTB	1609 w	770 w	706 m

* The designations of bands are given by KATRITZKY and AMBLER (65)
w = weak
m = middle

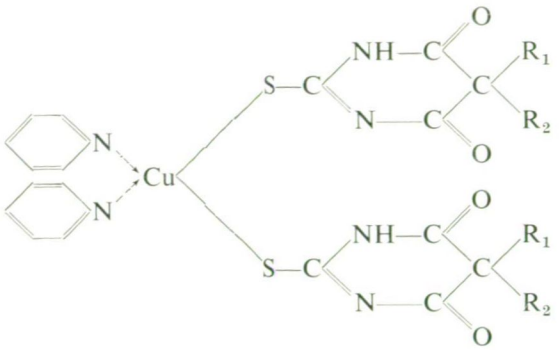
Table 13

Absorptions characteristic of the thiobarbiturate in the pyridinoccopper (II) thiobarbiturate complexes

	N–H (cm^{-1}	C=O (cm^{-1})
5-allyl-5-(2-methylpropyl) thiobarbituric acid AMPTB	3500, 3180	1705, 1640
5-ethyl-5-(1-methylbutyl) thiobarbituric acid EMBTB	2500, 3230	1690, 1640
5-ethyl-5-(1-methylpropyl) thiobarbituric acid EMPTB	3500, 3180	1705, 1640
5-allyl-5-(1-cyclohexenyl) thiobarbituric acid ACHTB	3500, 3200	1708, 1645

Among the νC=X bands appears the Amide I band of the $-NH-\overset{|}{C}=O$ group, while in the region of 1640 cm^{-1} the Amide I band of the $\diagdown C=N-\overset{|}{C}=O$ grouping is to be found. The intensity of the latter is roughly inversely proportional to the intensity of the νN–H stretching bands appearing in the region of 3500 cm^{-1}. On the basis of the above, it is assumed that in the crystalline state the rings are linked together, probably by hydrogen bonds.

Considering the results obtained it seems reasonable to suggest the following general structure for our thiobarbituratopyridinocopper(II) complexes:



This suggestion is supported by the observations of BAGGESGAARD—RASMUSSEN and JERSLEV (4) who noted that this type of complex can be prepared only in the case of 5,5- and 5,5,N-substituted barbituric acid derivatives, because the N,N'-, the 5,N,N'- and the 5,5,N,N'-substituted derivatives failed to give colour reaction.

3.0. Photometric determination of thiobarbiturates

3.1. Photometric determination of thiobarbituric acid derivatives on the basis of their pyridinocopper(II) complexes

The structure of pyridinocopper(II) thiobarbiturate complexes was determined (95, 104) and their properties studied (Chapters 2.2, 2.3, 2.4, 2.5, 2.6, 2.7) they seemed to be suitable for photometric determinations. These complexes are green, whereas the corresponding barbituric acid derivatives violet; consequently they can be readily distinguished from each other.

According to GOMAHR and KRESBACH (50) the copper(II) complexes are more stable than those of cobalt(II), and thus their photometric determination is much simpler. We ourselves applied the cooper(II) ion (100), RAVENTOS (116) copper(II) sulphate and diethylamine, and HEISE and KIMBEL (53) pyridinocopper(II) for the determination of thiobarbiturates.

The latter authors performed the determination by extracting the coloured complex with chloroform. They found the chloroform phase to be brown with Trapanal, brownish-green with Thiogenal, yellow with Inactin, and olive-green with Baytinal. Since such differences in the spectra of these compounds are not expected as a result of their structural similarity, we repeated the procedure with the pharmaceuticals listed above. The result was a green colour in each case. To increase the colour sensitivity, HEISE and KIMBEL (53) added solid potassium hydroxide to the mixture and then obtained the absorption spectra of the compounds. From these they established that homogeneous complexes were formed and that the green colour was not produced by the superimposition of the known blue colour, of the ZWIKKER reaction (159) and some secondary yellow colour. Details of the quantitative determination and the concentration curve were not reported.

GOMAHR and KRESBACH (50) investigated reactions of different substances, among them thiobarbiturates, with copper(II) sulphate. The mixtures were shaken with chloroform/pyridine (9:1) and the colour formation studied, but a quantitative determination was not reported.

Barbiturates have been determined by means of pyridinocopper(II) complexes by several authors (42, 46, 53, 66, 116).

3.1.1. Establishment of the specificity of the reaction

First of all we studied several pharmaceuticals which give a colour reaction with the pyridinocopper(II) reagent (5×10^{-2} M copper(II) sulphate in 15:85 pyridine:water (v/v); pH 7.4.). For the photometric determination the quantity of reagent added was slightly in excess of that calculated on the basis of the expected complex formula;

it was sufficient for complex formation of 0.005—0.025 g of the organic substance. 2 ml of the pyridinocopper(II) reagent was used containing 6.35 mg of Cu(II) and 294 mg of pyridine. The results can be seen in Table 14.

Table 14

Colour reactions of several pharmaceuticals with pyridinocopper(II), reagent and the possibilities of extracting the colours into chloroform

Substance	Solvent	Colour	Colour of the chloroform phase
Acetylsalicylic acid	water and methanol	blue	blue
Ascorbic acid	water	greenish-yellow	colourless
Caffeine	water and pyridine	blue	,,
Theophylline	boiling water	greenish-blue	brownish-green
Theobromine	,, and pyridine	blue	colourless
Procaine hydrochloride	water	,,	,,
Sulphathiazole	pyridine and water	violet-blue	greenish-grey
Sulphamethylthiazole	,,	violet	blue
Sulphadimethylpyrimidine	,,	green	green
Sulphathiocarbamide	,,	precipitate formed	turbid
Urethane	,,	blue	colourless
5,5-diethylbarbituric acid	pyridine and water	violet	violet
2-thiobarbituric acid	boiling water and pyridine	green	turbid
5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid	pyridine and water	,,	green

It is clear from the Table that only sulphadimethylpyrimidine gives the same colour reaction as thiobarbiturates. The chloroform phase is greenish-grey with sulphathiazole and brownish-green with theophylline. These latter, nevertheless, can easily be distinguished from the bright green colour of thiobarbiturates.

3.1.2. Influence of the pH and the pyridine concentration on the formation of pyridinocopper(II) thiobarbiturate complexes in various solvents

Since our previous experiments had showed that the formation of pyridinocopper(II) thiobarbiturate complexes strongly depended on the pH of the solution, on the concentration of copper(II), thiobarbiturate and pyridine, and on the solubility of the complex in a given solvent, experiments were made for the elucidation of these varying factors. We applied 50% aqueous methanol because in this solvent the complexes were extremely stable: the extinctions of the solutions were found to be unchanged even after several months.

As confirmation of all that has been said previously the following Figures show the influence of certain factors on the complex formation in the case of $Cu(II)(py)_2(EMBTB)_2$ as model.

As Fig. 36 shows, the $Cu(II)(py)_4$ complex is formed only in the 15, 20 and 25% pyridine solutions of 5×10^{-3} M copper(II) sulphate. A lower concentration of pyridine is insufficient for complete complex-formation. On acidifying with perchloric

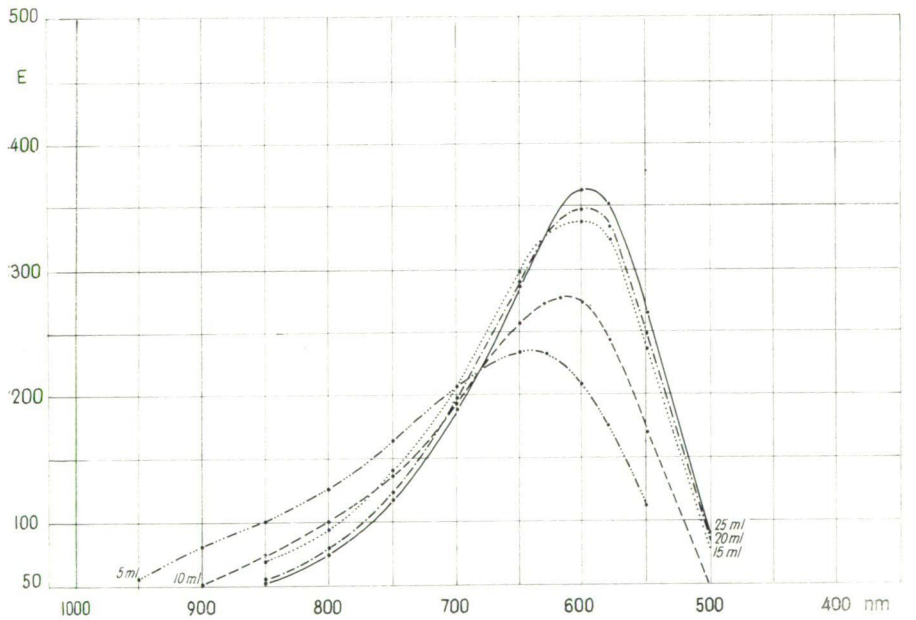


Fig. 36: Formation of $Cu(II)(py)_4$ as a function of pyridine concentration (5×10^{-3}M Cu^{2+}; pH 5.8).

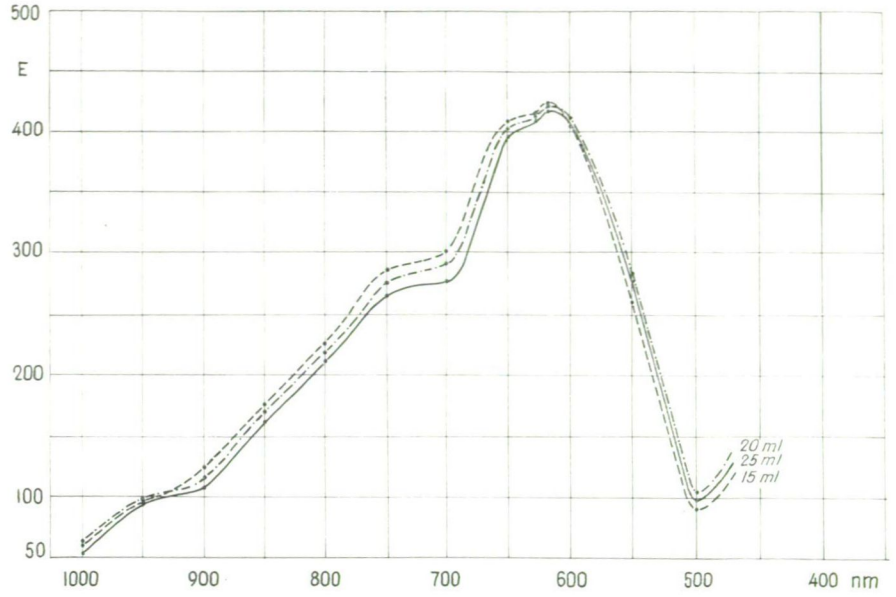


Fig. 37: Formation of $Cu(II)(py)_2(EMBTB)_2$ as a function of pyridine concentration (5×10^{-3} M Cu^{2+}; 10^{-3} M EMBTB; pH 6).

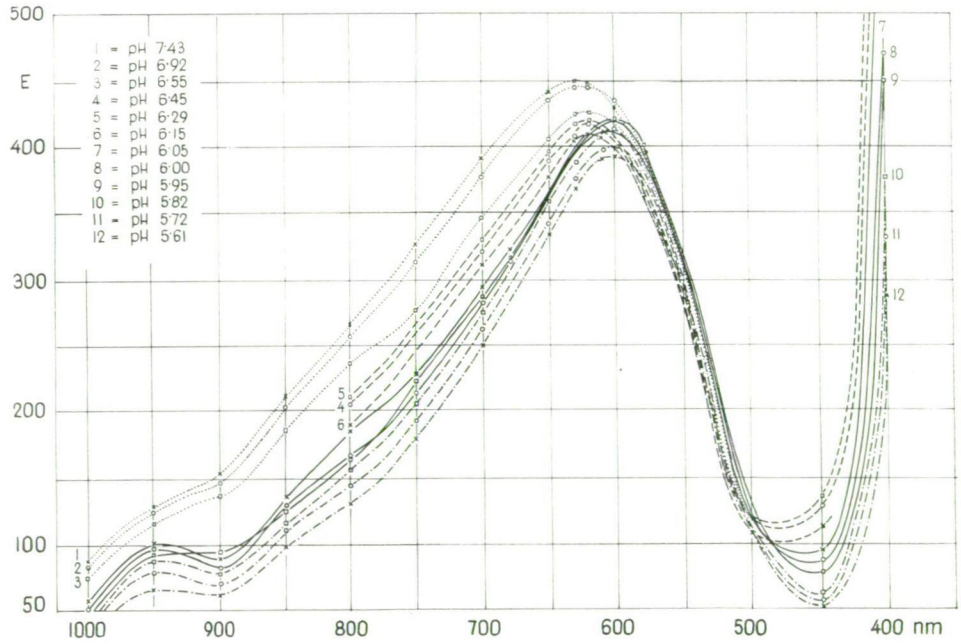


Fig. 38: Effect of pH on the formation of $Cu(II)(py)_2$ $(EMBTB)_2$ (5×10^{-3} Cu^{2+}; 1 M py; 10^{-3} M EMBTB).

acid the complex gradually becomes colourless below pH 5.8. Above pH 8 it decomposes and under the influence of the applied alkali copper(II) hydroxide precipitates.

Fig 37 shows that the quantity of pyridine added at pH 6 had no effect on the absorption maximum at 625 nm. Hence 15% pyridine was sufficient for complete complex formation. At pH 7.42 the situation is the same. The absorbance at the maximum increases linearly with increasing quantity of thiobarbituric acid. Thiobarbituric acid, nevertheless, should not be present in a higher molar ratio than 1 thiobarbiturate:5 pyridino copper(II) reagent.

Fig. 38 shows the absorption spectrum of pyridinocopper(II) thiobarbiturate measured at different pH's. The maximum lies at 625 nm from pH 6 upwards. The highest extinction values can be measured between pH 6.92 and 7.43.

From the above experiments it is clear that the complexes can be conveniently applied for quantitative determination only when they are extracted or the excess of the reagent is removed. Otherwise, significant differences can be observed in the absorption as a result of the pH effect.

Reagents required for photometric determinations.

Aqueous solution of 5×10^{-2} M copper(II) sulphate containing 15% pyridine
1 M potassium hydroxide
Distilled water:pyridine (3:2)
Chloroform p. a.
Benzene p. a.

3.1.3. Photometric determination of thiobarbiturates as pyridinocopper(II) mixed complexes by chloroform extraction

A measured amount of thiobarbiturate was dissolved in chloroform. From the stock solution prepared in this way a quantity corresponding to 5—25 mg was measured and made up to 5 ml with chloroform.

To this solution 2 ml of pyridinocopper(II) reagent was added, the mixture shaken, and the lower layer separated. Shaking was repeated with chloroform (2.5 and 2 ml), then the combined phases were made up with chloroform to 10.0 ml. The resulting solution is not opalescent and its colour intensity is quite definite. The extinction of the green colour of the complexes was measured in a 1 cm cell by means of a spectrophotometer. The maximum value of the extinction spectrum was found at 625 nm.

The colour stability of the complexes formed was checked by measuring the extinction in a covered cell at different intervals. The resulting values are collected in Table 15.

In connection with the examination of barbiturates, MATTSON and HOLT (82) report that the colour of the pyridinocopper(II) complex is stable for several hours. Nevertheless, from our results it appears that after half an hour the extinction of the thiobarbiturate mixed complexes shows a tendency to decrease slowly. The colour intensity was quite stable for an hour but the presence of water caused a decrease of the colour intensity and an opalescence. For the experiments the optimum laboratory temperature was 20 °C. We attempted to extract the pyridinocobalt(II)

Table 15

Colour stability of pyridinocopper(II) thiobarbiturate complexes in chloroform

Complex	Number of minutes before measurement of extinction				
	0	30	60	120	150
Cu(II) $(py)_2$ $(EMPTB)_2$	0.53	0.53	0.515	0.50	0.48
Cu(II) $(py)_2$ $(EMBTB)_2$	0.465	0.465	0.45	0.43	0.41
Cu(II) $(py)_2$ $(AMPTB)_2$	0.49	0.485	0.47	0.43	0.42
Cu(II) $(py)_2$ $(ACHTB)_2$	0.365	0.365	0.35	0.33	0.31

thiobarbiturate and pyridinonickel(II) thiobarbiturate complexes with chloroform but they were unsuitable for photometric measurements because of the unsatisfactory colour intensity and the opalescence of the resulting pink and pale blue solutions, respectively.

3.1.4. Photometric determination of thiobarbiturates by benzene extraction of their pyridinocopper(II) mixed complexes

Pyridinocopper(II) thiobarbiturate complexes can be extracted with benzene to give green solutions which can be used for photometric determination. No data are available in the literature concerning this finding.

On the basis of the previous method with chloroform the weighed thiobarbituric acid derivative was dissolved in benzene. From the stock solution an aliquot quantity corresponding to 5—25 mg of TB was taken out and made up to 5 ml with benzene. To this solution 2 ml of pyridinocopper(II) reagent was added and, after shaking, allowed to stand. The upper benzene phase was separated in a separating funnel or by means of a pipette and repeatedly shaken with benzene (2.5 and 2 ml, respectively). The combined benzene fractions were made up with benzene to 10.0 ml and the extinction of the green complexes was measured in a 1 cm cell by means of a spectrophotometer. The absorption maximum was again found at 625 nm, and consequently the solvent had no effect on the position of the maximum. The extinction was measured in a covered cell at different intervals, when it was found that the colour intensity did not change within half an hour (similarly to the chloroform method), but later did show a tendency to decrease. In the extraction methods there is a linear correlation between the extinction and the concentration; these procedures are most suitable for the determination of 0.005—0.020 g of thiobarbiturate. These methods are direct and quick; waiting time is not necessary. As a result of the suitable sensitivity and colour stability of the complex it can be favourably applied for determination of the thiobarbiturate content.

3.1.5. Photometric determination of thiobarbiturates by means of their pyridinocopper(II) mixed complexes after alkaline decomposition of the excess reagent

In addition to the former procedures, another method was developed for the photometric determination of pyridinocopper(II) thiobarbiturate mixed complexes. When a pyridinocopper(II) complex reacts with potassium hydroxide solution the complex decomposes and copper(II) hydroxide precipitates. The precipitate is a characteristic blue and the solution colourless if enough potassium hydrosyde is present. On the other hand, the pyridinocopper(II) thiobarbiturate mixed complex dissolves in potassium hydroxide to give a green solution. In the case of an appropriate amount of alkali the excess pyridinocopper(II) reagent decomposes and separates as copper(II) hydroxide. After centrifugation, the resulting green solution can be used for photometric determination.

In the course of our investigations the thiobarbiturates were dissolved in pyridine and diluted with water to obtain an aqueous solution containing 40% pyridine. The concentration of thiobarbiturate in the pyridine-water solvent was approximately 2×10^{-2} M; samples equivalent to 5—25 mg of TB were removed and each was made up to 5 ml with a water:pyridine (3:2) mixture. After addition of 2 ml of pyridinocopper(II) reagent to the mixture, it was shaken, allowed to react with 3.0 ml of M potassium hydroxide and finally centrifuged. The extinction of the clear green centrifuged solution was measured in a 1 cm cell with a spectrophotometer. The absorption maxima of the samples were at 625 nm.

The sodium salts of thiobarbituric acid can be directly determined photometrically by this method. When using this procedure sodium thiobarbiturate was dissolved in 3 ml of water:pyridine mixture (3:2) and the solution made up to 5 ml with the same mixture.

From the stock solution quantities equivalent to 5—25 mg were removed and, after making up to 5 ml, 2 ml of pyridinocopper(II) reagent was added to the shaken solution. 3 ml of 1 M potassium hydroxide was added, the mixture again shaken, and, after being kept 1—2 minutes, centrifuged for 7 minutes. The extinction of the clear green solution thus obtained was measured with a Spektromom 201 spectrophotometer. The absorption spectrum is quite similar to those of thiobarbituric acid derivatives; its maximum was found to be at 625 nm.

Table 16

Photometric determination of EMBTB by chloroform extraction of its pyridinocopper(II) complex

Stock solution ml	$CHCl_3$ ml	Pyridinocopper(II) reagent	$CHCl_3$ extr. ml	Concentration g/10 ml	Extinction
1	4	2 ml	2.5+2.0+0.5	0,004846	0,15
1,5	3,5			0,007269	0,225
2	3			0,009692	0,315
3	2			0,014538	0,475
4	1			0,019384	0,61
5	0			0,024230	0,745
5,5	0		2,5 2,0	0,026653	0,835

Table 17

Photometric determination of EMBTB by benzene extraction of its pyridinocopper(II) complex

Stock solution ml	Benzene ml	py-Cu(II)-reagent	Benzene ml	Concentration g/10 ml	Extinction
0,5	4,5			0,002304	0,07
1	4			0,004608	0,14
1,5	3,5			0,006912	0,22
2	3			0,009216	0,30
2,5	2,5	2 ml	2,5 + 2,0 + 0,5	0,011520	0,38
3	2			0,013824	0,45
3,5	1,5			0,016128	0,52
4	1			0,018432	0,60
5	0			0,023040	0,74

Table 18

Photometric determination of EMBTB on the basis of its pyridinocopper(II) complex after alkaline decomposition of the excess pyridinocopper(II) reagent

Stock solution ml	Water-pyridine ml	py-Cu(II)-reagent	M KOH ml	Concentration g/10 ml	Extinction
0,5	4,5			0,002424	0,165
1	4			0,004848	0,29
2	3			0,009696	0,45
2,5	2,5	2 ml	3	0,012120	0,515
3	2			0,014544	0,615
3,5	1,5			0,016968	0,69
4	1			0,019392	0,78
5	0			0,024240	0,88

Table 19

Photometric determination of EMBTB-sodium salts on the basis of its pyridinocopper(II) complex, after alkaline decomposition of the excess pyridinocopper(II) reagent

Stock solution ml	Water-py ml	py-Cu(II) reagent	M KOH ml	Concentration g/10 ml	Extinction
0,5	4,5			0,001770	0,09
1	4			0,003540	0,22
2	3			0,007080	0,37
2,5	2,5	2 ml	3	0,008850	0,45
3	2			0,010620	0,53
3,5	1,5			0,012390	0,635
4	1			0,014160	0,72
5	0			0,017700	0,82

By means of the method described above EMBTB, EMPTB, AMPTB, ACHTB, and their sodium salts were determined (accuracy: within 2%).

Our investigations showed that the thiobarbiturates and pyridinocopper(II) complex sodium salts can be photometrically determined by the above procedure. The dependence of the extinction on the concentration is linear and suitable for determinations between 0.005—0.025 g. On standing, the colour intensity slowly decreases; therefore, determination must be performed immediately after centrifugation. To carry out this measurement, a laboratory temperature of 20 °C or less is required.

Extinction values are presented with EMBTB as model.

3.1.6. Evaluation of the results

On the basis of our examinations it was established that the pyridinocopper(II) complexes of thiobarbiturates were suitable for photometric determination.

Extraction with chloroform or benzene provides a simple means of separation of the complexes. Determination after alkaline decomposition of the excess reagent, however, makes possible the direct photometric determination of the sodium salts of thiobarbiturates. This is very important from the point of view of practical drug analysis because injections can thus be directly determined without transformation into the corresponding acid.

The Bouguer-Lambert-Beer law was valid for the system, and photometric measurement could be successfully applied for the determination of thiobarbiturates between 0.005 and 0.025 g. Advantages of the above methods are as follows:

1. they are simple and can be carried out easily;
2. due to their sensitivity and accuracy they are suitable for drug analysis;
3. there is no waiting time, so determinations are rapid;
4. a violet complex is formed with barbiturates and this excludes the possibility of the co-measurement of barbiturates.

A disadvantage of the method is that the reaction is the same with all the thiobarbiturates; thus, as in the case of mixtures, all the thiobarbiturates are measured together. Hence there is no possibility of distinguishing their small structural differences in this way.

Though thiobarbituric acid itself gives a green colour with the pyridinocopper(II) reagent, it cannot be measured photometrically because the colour intensity does not obey the Bouguer-Lambert-Beer law and it cannot be extracted by chloroform or benzene. It is clear from the preceding experiments that substituents on C-5 influence the characteristics of the complexes.

3.2. Use of thiobarbituratocopper(II) complexes for the photometric determination of thiobarbiturates

According to our investigations described in Chapter 3.1. the colour intensity of $Cu(II)—(py)_2—(TB)_2$ complexes is influenced by the quantity of amines and pyridine. Thus we attempted to determine thiobarbiturates also as their copper(II) complexes, without pyridine (100).

The sodium salts of the EMPTB, EMBTB, and ACHTB have been used for the experiments. From these derivatives 10^{-2} M stock solutions were prepared in distilled absolute ethanol since in this medium the precipitation of a complex was not experienced. The only difference was with the determination of the alcohol content necessary for the reaction. 10^{-2} M $Cu(NO_3)_2 . 3H_2O$ (Reanal p. a.) was prepared in absolute ethanol and used as reagent. Measurements were carried out in a thermostated Beckman DU and a Spektromom 360 spectrophotometer.

In order to find the most suitable experimental conditions, the following experi-

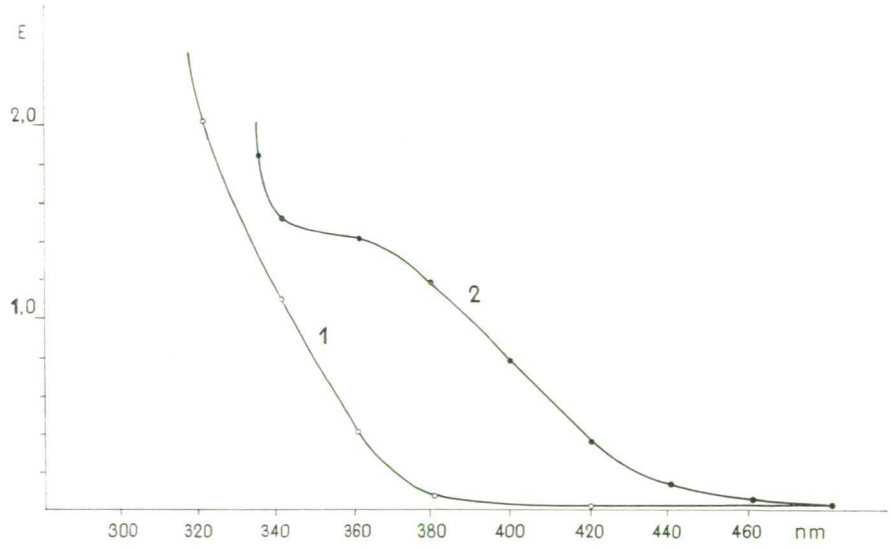


Fig. 39: Spectra of copper(II) nitrate (1) and copper(II) nitrate and different EMBTB Na(2). (1) 4×10^{-3} M $Cu(NO_3)_2 \cdot 3\ H_2O$, (2) 4×10^{-3} M $Cu(NO_3)_2 \cdot 3H_2O$; 3.7×10^{-4} M EMBTB in abs. ethanol

ments were carried out: the absorbances of solutions containing copper(II) nitrate, and copper(II) nitrate and different TBNa were measured (Fig. 39).

As appears from Figure 39, copper(II) forms a complex with TBNa. Spectra of the solutions were taken after an hour and no change in the absorbance could be found.

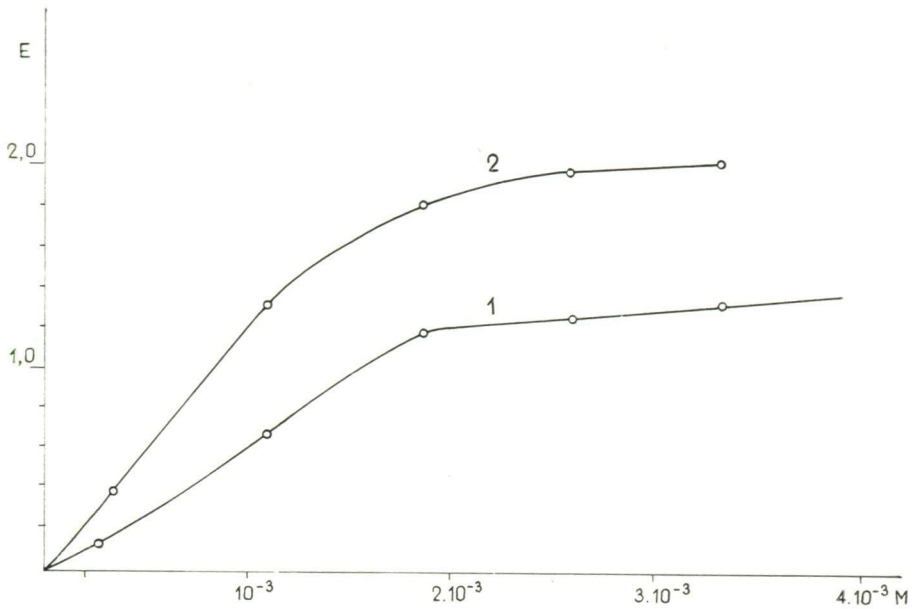


Fig. 40: Determination of the composition of the copper(II)-EMBTB complex by the molar ratio method. 10^{-3}—4×10^{-3} M EMBTB; 4×10^{-4} M $Cu(NO_3)_2\cdot3\ H_2O$ in abs. ethanol (1): $\lambda=420$ nm (2): $\lambda=400$ nm

The composition of the copper(II)-TB complex was determined by the molar ratio method; maintaining the metal ion concentration constant, the TBNa concentration was altered and the extinction of the solution measured at different wavelengths (Fig. 40).

Figure 40 shows that in absolute ethanol four 5,5-substituted thiobarbituric acid derivatives react with one copper(II) ion, thus the composition of the complex is: $Cu(II)(TB)_4$.

The effect of excess concentration of Cu(II) ions on the absorbance of the $Cu(II)(TB)_4$ complex has also been studied (Fig. 41).

The above Figure shows that the excess concentration of Cu(II) ions does not affect the absorbance of the complexes.

The effect of water on the absorbance of the Cu(II) $(TB)_4$ complexes in absolute ethanol has been investigated too (Fig. 42).

It appears from the Figure that in 50% ethanol the absorbance changes (there is still no complex precipitation), which means that on applying non-absolute alcohol a separate calibration curve must be prepared. It was also studied whether or not the absorbance of the $Cu(II)(TB)_4$ complex in absolute alcohol changes proportionally to the concentration, i. e. whether it follows the Bouguer-Lambert-Beer rule

at 360 nm (Fig. 43). This wavelength was chosen since it is here that the absorbances of $Cu(II)(TB)_4$ and Cu(II) nitrate exhibit the largest deviation. The Figure demonstrates well that the concentration curve is a straight line, indicating that the $Cu(II)(TB)_4$ complex is suitable for spectrophotometric determination. It is best to apply aliquots of $10^{-5}-7\times10^{-5}$ μg/ml thiobarbituric acid derivative when the extinction is in the measurement range of 0.1—0.9.

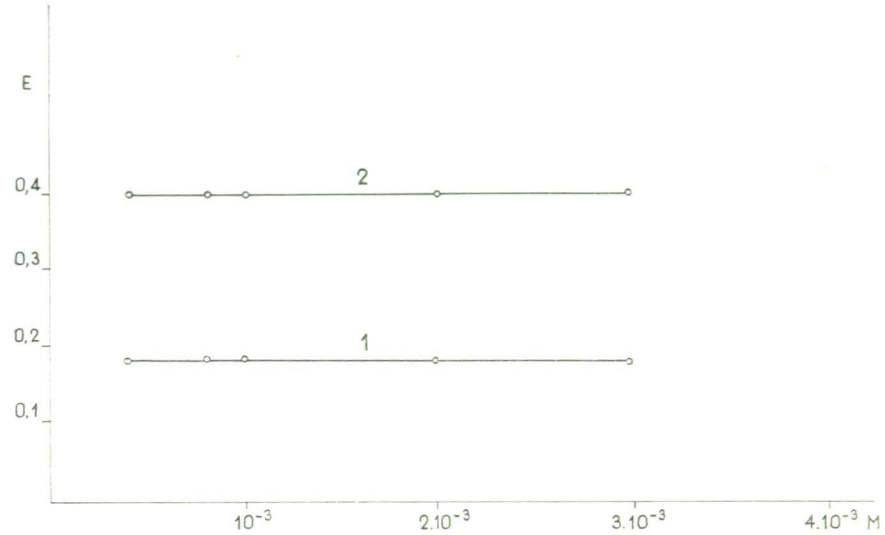


Fig. 41: Influence of the Cu(II) ion concentration on the absorbance of $Cu(II)(TB)_4$ (1): $\lambda=420$ nm 3.7×10^{-4} M ACHTB (2): $\lambda=400$ nm 3.7×10^{-4} M ACHTB

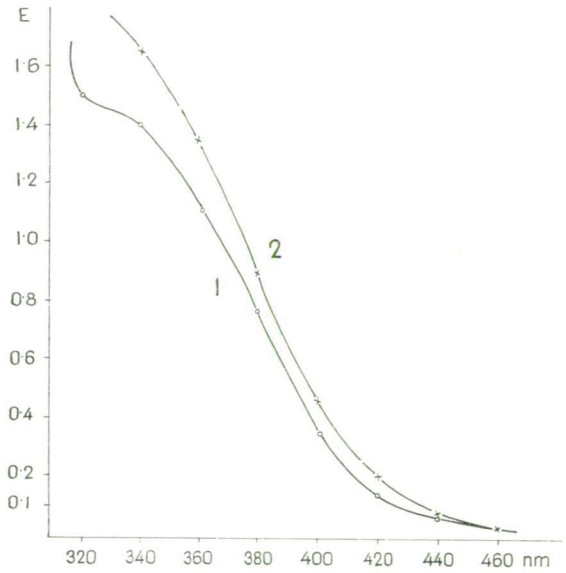


Fig. 42: Effect of water on the absorbance of $Cu(II)(TB)_4$ complexes in absolute ethanol. 4×10^{-3} M Cu^{2+}; 3.7×10^{-4} M ACHTB (1): 100% ethanol (2): 50% ethanol

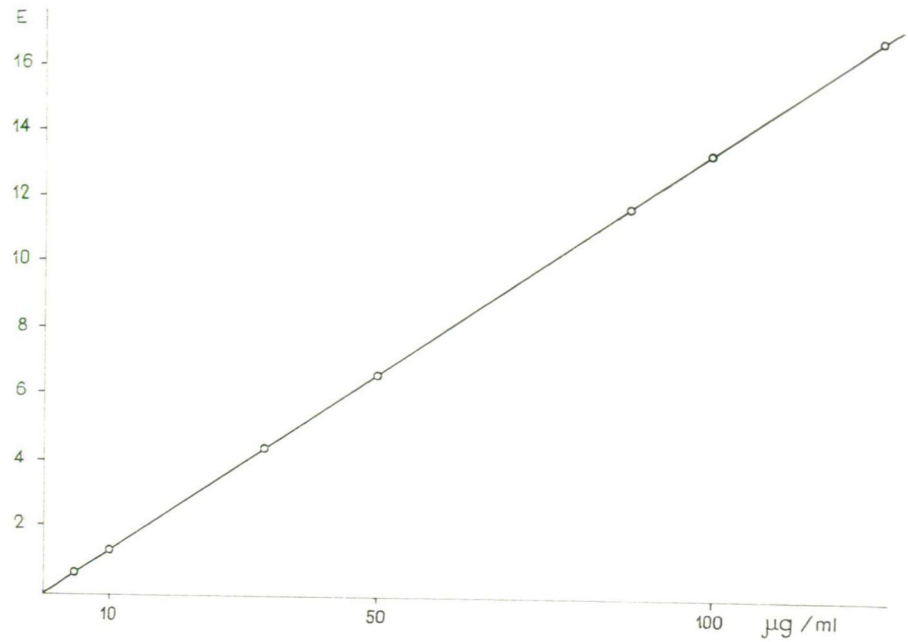


Fig. 43: Calibration curve of EMBTB in ethanol; 360 nm; cell 1 cm.

Table 20

Spectrophotometric determination of EMBTB in form of Cu(II) $(TB)_4$ complexes, at 360 nm

TBNa	Taken (µg)	Found (µg)	Difference (△ µg)	Deviation %
EMBTB	50	50,2	+0,2	+0,4
	50	50,0	0,0	0,0
	75	74,7	—0,3	—0,4
	75	74,5	—0,5	—0,7
	100	100,1	+0,1	+0,1
	100	99,9	—0,1	—0,1
	110	110,0	0,0	0,0
	110	110,5	0,5	+0,4

Accuracy: within ± 1%

Measurements were performed in the following manner: 20 mg of the sodium salt of the thiobarbituric acid derivative to be determined was weighed by difference (hygroscopic compound) into a 100 ml volumetric flask, dissolved in absolute ethanol and filled up to the mark. 2 ml were pipetted into a 25 ml volumetric flask to which 10 ml absolute ethanolic copper(II) nitrate solution had previously been added. Thereafter it was filled up to the mark with absolute ethanol, thoroughly shaken and measured in a 1 cm cell in the spectrophotometer at 360 nm. Results were read from a previously prepared calibration curve. The method has been successfully applied for the determination of sodium salts and injections of thiobarbituric acid derivatives provided as dry powders in sealed ampoules. Our results are listed in Table 20.

3.3. Photometric determination of thiobarbiturates as their cobalt(II) thiobarbiturate complexes

The PARRI reaction (106) of barbituric acid derivatives in the form of their cobalt complexes has been employed by a number of authors (1—4, 7, 15, 42, 50, 51, 64, 70, 75, 81, 110, 134) for analytical purposes. Nevertheless data concerning the determination of thiobarbiturates can seldom be found. HEISE and KIMBEL (53) used sodium nitroprusside to determine thiobarbiturates photometrically; in their work they reported the determination of Inactin and Thiogenal in addition to different methionine derivatives, using the colour reaction with cobalt(II) ion. Considering that the cobalt complexes of thiobarbiturates seemed to be suitable for the determination of other members of this group, we have studied this problem in detail.

Methanolic solutions of Co(II) thiobarbiturate and thiouracil complexes are green, while that of barbiturates is violet. Hydantoins, and ureides of glycoleic acid complexes, form blue complexes with the cobalt(II) ion in methanol. This difference in colour is one of the advantage of the PARRI reaction.

Concerning the composition of the barbiturate complexes, data can be found in ZWIKKER's publication (160) according to which the Co:barbiturate ratio in the complex is 1:2 (anhydrous methanolic medium). This ratio remains unchanged when the solution is made alkaline with calcium hydroxide or barium hydroxide. SCHWENKER (130) obtained the same result when he carried out the complex reaction in alkaline methanol.

We extended our investigations to EMBTB, AMPTB and ACHTB, most frequently used in therapy, and to their sodium salts. Starting materials were analysed according to the PHARMACOPOEA INTERNATIONALIS, Ed. I. (112). Results obtained are listed in Table 21.

Table 21

Determination of thiobarbiturates and their sodium salts, according to the PHARMACOPOEA INTERNATIONALIS, I.

Thiobarbiturate (sodium salt)	Sodium content %		Thiobarbiturate content %	
	calcd.	found	calcd.	found
1. EMBTB	8.49	8.75	91.50	90.8
2. AMPTB	8.56	8.80	91.43	90.5
3. ACHTB	7.84	8.20	92.15	91.3

For the photometric measurements we used only undecomposed materials containing thiobarbiturate between the given limits. The purity of the thiobarbituric acids was checked by their melting points.

3.3.1. Determination of thiobarbituric acid derivatives

Reagents and stock solution:

1. methanolic cobalt(II) chloride solution saturated at room temperature
2. solid potassium hydroxide
3. 10^{-2} M thiobarbituric acid derivative in methanol

From the 10^{-2} M methanolic thiobarbituric acid stock solution increasing portions (beginning with 2.5 mg) were withdrawn, transferred into centrifuging tubes fitted with glass stoppers, made up to 5 ml with methanol, and 5 drops of saturated methanolic cobalt(II) chloride solution were added to each sample. Each was shaken, then ca. 0.2 g of potassium hydroxide (1 pellet) was added to the solution; it was again shaken, allowed to stand for 2 hours, and centrifuged at 3000 r. p. m. The absorption spectrum of the bluish green solution thus obtained was measured with a „Spektromom 360" photometer in a 1 cm cell referred to water as blank.

In contrast to HEISE and KIMBEL (53), we found the maximum of the absorption spectrum to be at 625 nm, and thus our photometric measurements were carried out at that wavelength.

By means of the method mentioned above we have plotted the concentration curves of EMBTB, AMPTB, and ACHTB. We found that cobalt complexes of the

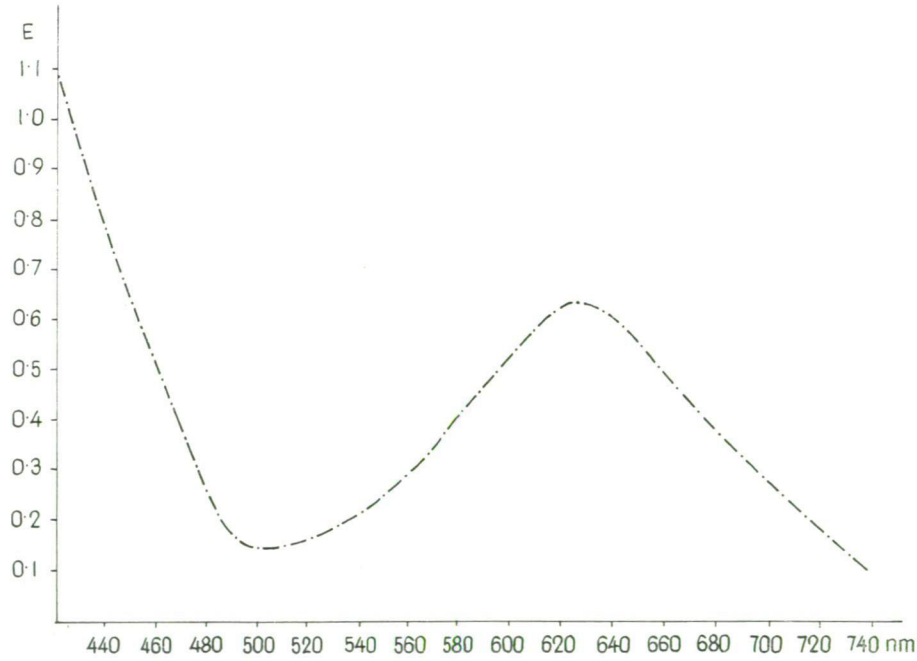


Fig. 44: Absorption curve of the cobalt(II)-EMBTB complex. (Concentration: 1.5×10^{-2} M)

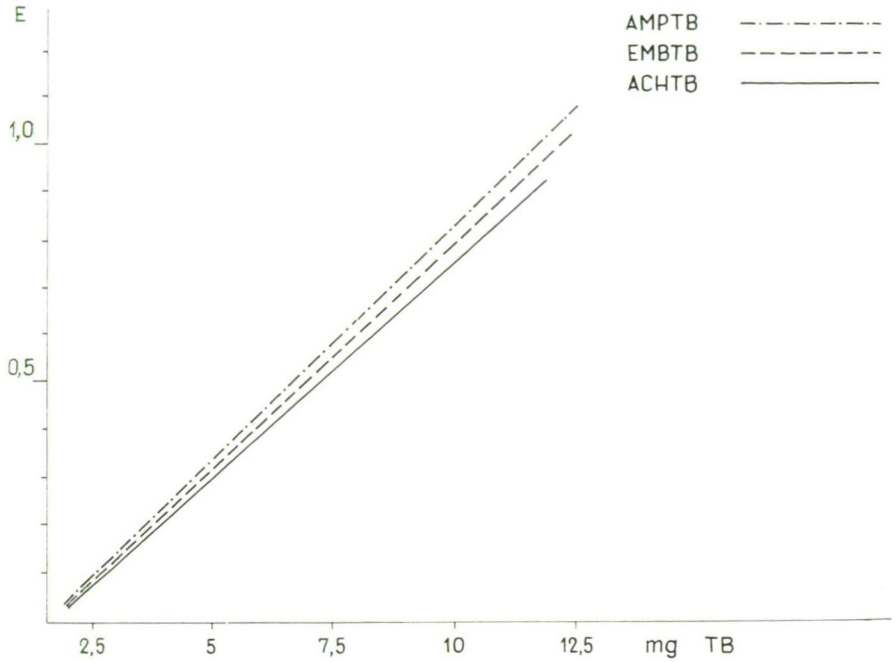


Fig. 45: Concentration curves of EMBTB, AMPTB, and ACHTB.

thiobarbituric acid follow the Bouguer-Lambert-Beer law between 10^{-2} and 10^{-3} M concentration limits; hence they are suitable for photometric determination.

Fig. 45 illustrates the results of our measurements.

The photometric procedure in the literature, based upon the complex formation of cobalt(II) thiobarbiturate, is suitable for the determination of the thiobarbituric acid derivatives. In practical drug analysis, however, it is the sodium salts of these compounds that must be determined. The latter compounds, applied as intraveneous narcotics, are dispensed as dry powders in sealed ampoules. That is why the direct determination of the sodium salts of the thiobarbituric acid derivatives is of importance.

3.3.2. Photometric determination of sodium salts of thiobarbituric acid derivatives by means of their cobalt(II) complexes

Sodium thiobarbiturates are hygroscopic and labile; consequently the injection must be freshly prepared, by dissolving the contents of the sealed ampoule promtly before use. To obtain correct results, it is also advisable to perform the determination immediately.

Water absorption of the sodium salts of thiobarbituric acid derivatives was studied on the sodium salt of EMBTB. The relative humidity of the air was 49.8% determined by means of a hygrometer with two thermometers („Labor" type). Fig. 46 demonstrates our results.

As the data of Fig. 46 show, it is advisable to carry out the measurement in a ground-glass stoppered measuring vessel immediately after opening the ampoule.

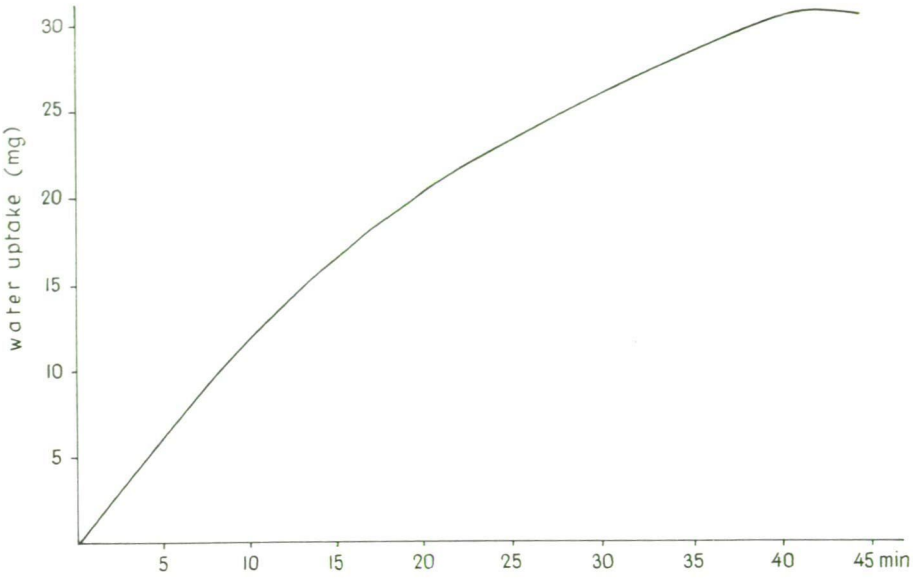


Fig. 46: Water absorption of sodium EMPTB at a relative humidity of 49.8% (starting weight: 0.4650 g).

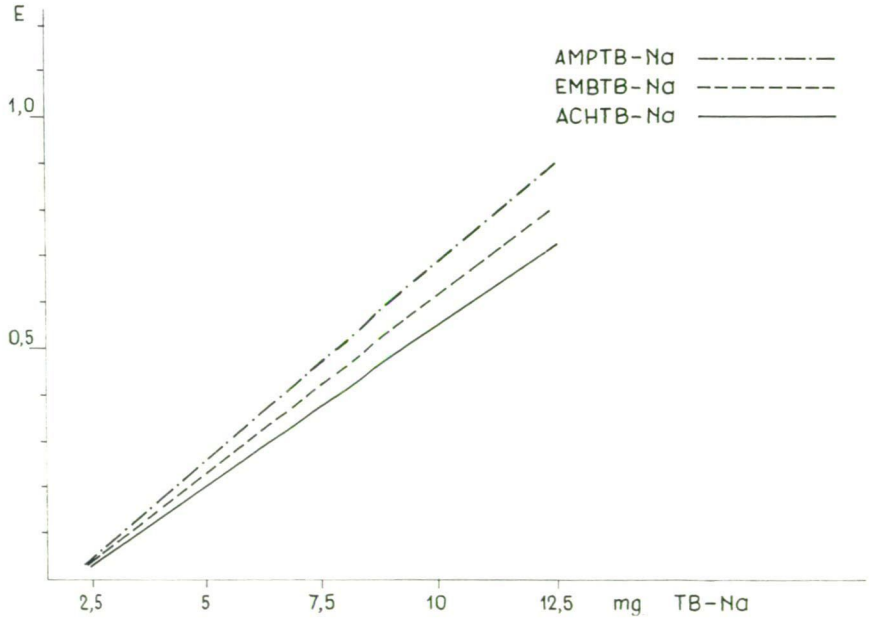


Fig. 47: Concentration curves of EMBTB, AMPTB, and ACHTB sodium salts from direct measurements.

The photometric determination was carried out as follows. The measured amount of sodium thiobarbiturate (ca. 0.08 g) was dissolved in 25.0 ml of methanol containing 10 drops of M HCl. From this stock solution increasing portions, containing from 2.5 to 12.5 mg, were withdrawn and measured into centrifuging tubes provided with stoppers, and made up to 5.0 ml with methanol. 5 drops of saturated methanolic cobalt(II) chloride were added dropwise to each. The reaction mixtures were shaken and about 0.2 g of solid potassium hydroxide was added; the mixtures were again shaken, allowed to stand for 2 hours, centrifuged, and the extinction measured in a 1 cm cell at 625 nm. The absorption spectra of the sodium thiobarbiturates were determined by the method described above for the corresponding acid. Their concentration curves give directly the values of the sodium salt of the corresponding thiobarbituric acid (Fig. 47).

We carried out some experiments with methanolic solutions of sodium thiobarbiturates and cobalt(II) chloride by means of a photometric method in order to establish the time and conditions required for complex formation.

The spectra of the solutions containing Co^{2+} and thiobarbiturates which were measured at different times are shown in Fig. 48.

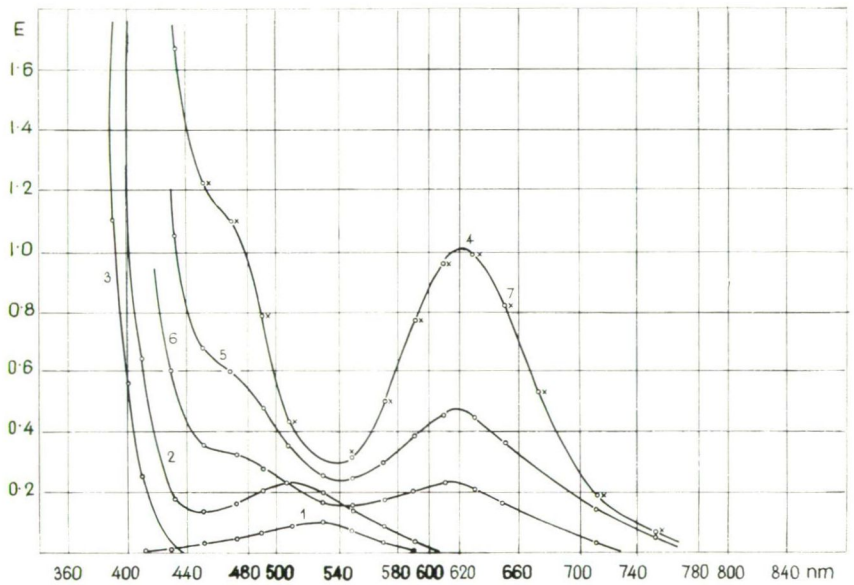


Fig. 48: Formation of cobalt(II)-EMBTB complex as a function of time and pH.
Curve 1: 1.2×10^{-2} M $CoCl_2\cdot6\,H_2O$,
Curve 2: 1.2×10^{-2} M $CoCl_2\cdot6\,H_2O$,
1.8×10^{-2} M EMBTB
Curve 3: 1.8×10^{-2} M EMBTB
Curve 4: 3×10^{-2} M $CoCl_2\cdot6\,H_2O$,
1.8×10^{-2} M EMBTB and 0.2 g KOH after standing for 2 hours
Curve 5: 6×10^{-3} M $CoCl_2\cdot6\,H_2O$,
2.7×10^{-2} M EMBTB-Na, after standing for 3 hours
Curve 6: 6×10^{-3} M $CoCl_2\cdot6\,H_2O$,
3.6×10^{-2} EMBTB-Na, after standing for 1 hour
Curve 7: 3×10^{-3} M $CoCl_2\cdot6\,H_2O$,
1.8×10^{-2} M EMBTB-Na, after standing for 24 hours

Curves 4 and 7 show that the reaction occurs more rapid in the presence of potassium hydroxide. On the other hand, when a methanolic solution of cobalt(II) and thiobarbituric acid is allowed to stand for 24 hours no green complex forms (Curve 2). Since no reaction takes place with thiobarbituric acid without making the solution alkaline (in the presence of potassium hydroxide it proceeds in several hours) we concluded that the reaction between sodium thiobarbiturate and cobalt(II) ions depends on the basicity of the solution.

Although it was possible to measure pH in a methanolic medium the strength of the colour reaction could be given approximately in the following way:

Co^{2+} + sodium thiobarbiturate + potassium hydroxide $\gg$ (stronger than) Co^{2+} + + sodium thiobarbiturate > (stronger than) reaction product from Co^{2+} + thiobarbituric acid.

3.3.3. Dependence of the reaction on time and temperature

The colour stability of the complexes formed was investigated by two methods in the case of thiobarbituric acid derivatives.

1. *Time dependence.* The extinction of the cobalt(II) complexes of thiobarbituric acid derivatives was measured at different times. The results of the investigation

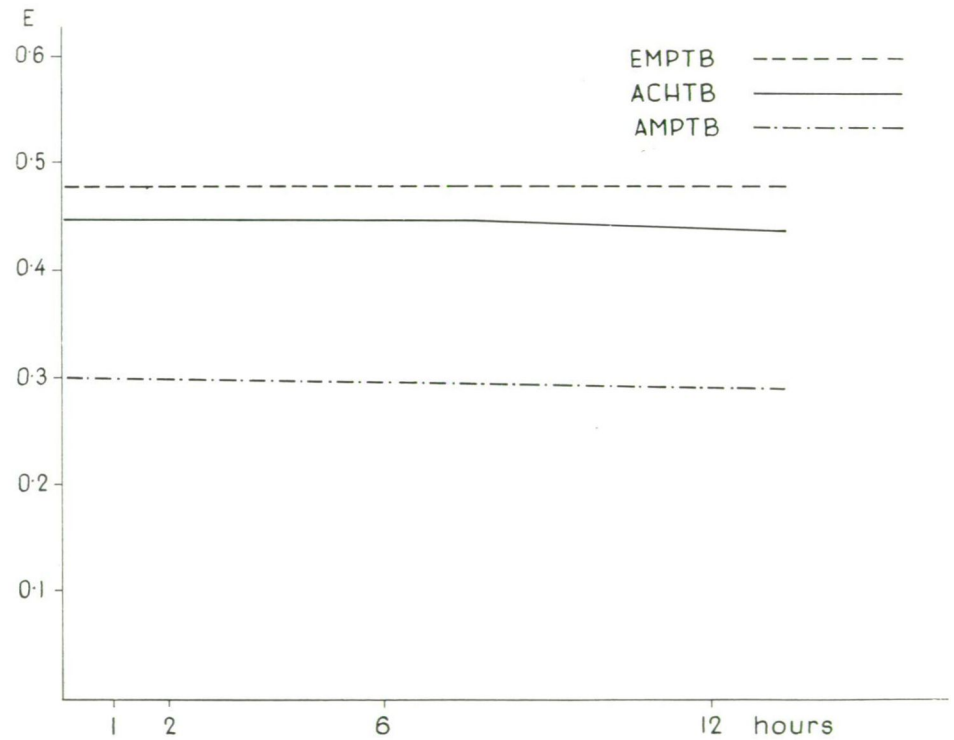


Fig. 49: Investigation of colour stability of cobalt(II) thiobarbiturate complexes as a function of time.

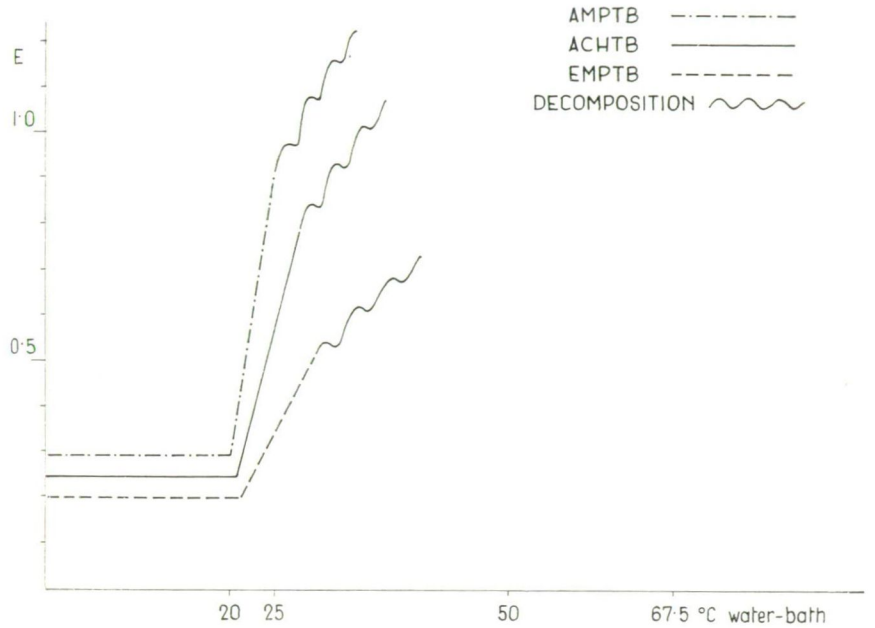


Fig. 50: Influence of the temperature on the colour stability of cobalt(II) thiobarbiturate complexes.

showed (Fig. 49) that the extinction remained unchanged within the limit of error after 12 hours; indeed, even after a much longer time it showed only a small decrease.

2. *Dependence on the temperature.* Solutions of the complexes were kept at different temperatures for an hour and then the extinction was measured.

After an hour at 5, 10, 15 or 20 °C the extinction was unchanged, but at 25 °C it increased significantly. When the temperature was further increased, the extinction value exceeded 1. This was the consequence of the decomposition of the complex since the solution became turbid at the same time. Therefore, it was important to keep the temperature at 20 °C or less during the investigations.

Our results are shown in Fig. 50.

By means of the above method the dry content of the ampoule (sodium thiobarbiturate), and the thiobarbituric acid derivatives also, can be simply and quickly determined.

4.0. Investigation of thiobarbiturates and pyridinocopper(II) thiobarbiturate mixed complexes by means of paper electrophoresis

Since thin layer chromatographic investigations of $Cu(II)(py)_2(TB)_2$ complexes were unsuccessful, we attempted to examine these compounds by paper electrophoresis.

Several authors have already dealt with paper electrophoresis of barbiturates, e. g. KINOSHITA and MORIYAMA (67), CALO et al. (19), STAINIER et al. (133), MERVILLE et al. (83), and TOMODA et al. (142). However, data concerning the electrophoresis of thiobarbiturates could not be found. Thus we attempted to develop a procedure suitable for the electrophoresis of thiobarbiturates and pyridinocopper(II) thiobarbiturate complexes. Our further aim was to adopt that method for the electrophoresis of thiobarbiturates in serum. This problem is very important because protein fractions of patients narcotized with thiobarbiturate must frequently be determined and this necessitates knowing the electrophoretic behaviour of thiobarbiturates.

The electrophoretic migration of pyridinocopper(II) thiobarbiturate mixed complexes was also completely unknown; therefore, we studied this question with the aim of furnishing further data concerning the properties of the complexes.

Thiobarbituric acid derivatives were prepared as in chapter 2.2, and the corresponding pyridinocopper(II) mixed complexes by our earlier reported method (95).

For the choice of the appropriate electrolyte we examined a number of solutions of different composition. We studied the correlation of the voltage increase with the time period and with the ionic strength of the electrolyte for the respective buffers. The results obtained are listed in Table 22.

The Kolthoff buffer with pH 9.7 proved to be the most suitable. Using Tris solution, the applied substance could be developed neither with mercury(I) nitrate nor with silver nitrate, nor in iodine vapour. In the case of sodium citrate and ammonium hydroxide the development was also unsuitable. During the migration in the case of the disodium hydrogen phosphate buffer the bands of the complexes extended over 3—4 cm.

We applied the wet cell procedure of paper electrophoresis, i. e. paper strips impregnated with buffer solution are freely suspended horizontally between the two vessels containing buffer. A solution prepared from sodium dihydrogen phosphate and sodium tetraborate according to Kolthoff was used as buffer (adjusted to pH 9.7 with 2 M sodium hydroxide). From Whatman No. 1 paper ten strips were prepared in parallel (2.5 cm×20 cm) and at each edge one strip (1.5×20 cm). The strips were separated from each other by distances of 1.5 mm. The paper was wetted by immersion in the buffer and the excess buffer blotted off.

Table 22

Correlation between the voltage increase, the running time, and the ionic strength

Buffer	Initial voltage (V)	Final voltage (V)	Initial current (mA)	Final current (mA)	Running time
Kolthoff p_H 9,7	120	115	8	12	12^h
Ammoniumhydroxide	120	118	2	3	11^h
Kolthoff p_H 9,7	200	190	20	35	4^h
Kolthoff p_H 9,7 (2 steps)	200	190	23	40	4^h
Tris	250	245	3	5	6^h
Na_2HPO_4	250	250	12	15	1^h
Kolthoff p_H 9.7	300	295	20	32	$1^1/_2{}^h$

A fresh 1% solution was prepared from the thiobarbiturates and their pyridinocopper(II) complexes with 0.1 M sodium hydroxide. 100 μg of the substance, together with 0,1 ml 5×10^{-2} M pyridinocopper(II) reagent were applied to the start-line by means of a micropipette over the whole width of the paper strip. The products could be well detected when as little as 50 μg of substance was applied. As solvent we attempted to use pyridine but in this case the substances failed to migrate.

For direct control of the migration, dyestuff mixtures were run on both edges (fluorescein, phenol red, bromophenol blue, and methyl red). Together with the dyestuff mixtures, albumin or dextran were applied to establish the mobility values. On the basis of HUGHES's investigations (56), the electrophoretic mobility of albumin is (u) — 6.0×10^{-5} cm/volt sec., and that of dextran is zero because it has no charge.

After the run the paper was dried at 110 °C in a desiccator. Room temperature drying caused the developed spot to spread to a small extent.

As developing agents the following substances were used:

1. 1 per cent $Hg_2(NO_3)_2$ solution,
2. 0.1 M $AgNO_3$ and 10% NH_4OH (1:1),
3. I_2 vapour,
4. a solution of 0.5 g $CuSO_4$. 5 H_2O, 3 ml of diethylamine, and 100 ml of 10% NaOH for development of TB,
5. 10% $K_4[Fe(CN)_6]$ to develop copper.

For the increase of sharpness and intensity of the spots we elaborated a new developing method with mercury(I) nitrate which gave the most favourable result.

When dry, the paper was immersed in 1% mercury(I) nitrate solution for 1—2

minutes, then placed into 0.1 M sodium hydroxide solution. In the alkaline medium the spot became stronger and at the same time the paper turned grey. After reaction for 1—2 minutes, the paper was placed in a 0.5% nitric acid solution, then, after 3—4 minutes, removed and dried. On the whitened paper some well-defined, dark grey spots could be seen corresponding to thiobarbiturates and pyridinocopper(II) thiobarbiturate complexes. This method can be well applied for developing the thiobarbiturate content of serums on paper and agar-gel as well. In our method, the thiobarbiturate and the albumin ran approximately together; this was earlier proved using a product coloured with fuchsin.

Results of the investigations are summarized in Table 23.

Table 23

Investigation of thiobarbiturates and their pyridinocopper(II) complexes by electrophoresis

Applied substance (100 μg)	Buffer	Running time (h)	V	mA	Developing agent	Electrophoretic mobility (cm^2/volt sec)
EMPTB	KH_2PO_4—$Na_2B_4O_7$ Kolthoff buffer, p_H 9,7	4	200	24	1% mercury(I) nitrate solution	$-6{,}34.\ 10^{-5}$
Cu(II) $(py)_2$—$(EMPTB)_2$		4	200	24		$-7{,}33.\ 10^{-5}$
EMBTB		4	200	24		$-6{,}21.\ 10^{-5}$
Cu(II) $(py)_2$—$(EMBTB)_2$		4	200	24		$-6{,}94.\ 10^{-5}$
AMPTB		4	200	24		$-6{,}26.\ 10^{-5}$
Cu(II) $(py)_2$—$(AMPTB)_2$		4	200	24		$-7{,}46.\ 10^{-5}$
ACHTB		4	200	24		$-6{,}17.\ 10^{-5}$
Cu(II) $(py)_2$—$(ACHTB)_2$		4	200	24		$-7{,}03.\ 10^{-5}$
Cu(II) $(py)_4$		4	200	24	$K_4[Fe(CN)_6]$	—

From the data obtained it is clear that thiobarbituric acid derivatives and pyridinocopper(II) thiobarbiturate complexes migrate under the influence of electric current. The pyridinocopper(II) reagent remains on the start-line and the copper can be developed with potassium ferrocyanide. The migration rate of the complexes is always greater than that of the thiobarbituric acid derivatives.

During the run the yellowish band of pyridinocopper(II) thiobarbiturate complexes can be well seen. The freshly prepared complex applied on the whole width of the paper runs as a homogeneous, defined spot. The yellowish green band, after development with mercury(I) nitrate, corresponds to the strongest uppermost spot. In the case of $Cu(II)(py)_2(ACHTB)_2$, the yellowish green band spreads to an extent depending on the time between the preparation and application of the complex.

As was to be expected thiobarbiturates and their pyridinocopper(II) complexes failed to give great differences in electrophoretic mobility owing to the similarity

of their structures. Thiobarbiturates, however, could be well distinguished from serum thus in a simple way. On the other hand, it was proved that pyridinocopper(II) thiobarbiturate complexes did not decompose under the influence of the current and they did migrate. The corresponding thiobarbituric acid and its pyridinocopper(II) complexes could be submitted to electrophoresis with an electrophoretic mobility difference of $-1-1.3\times10^{-5}$ cm^2/volt sec.

5. SUMMARY

The present work is summarized in the order of Chapters; the results obtained are emphasized. (References: 93—100, 104)

The following compounds frequently used in therapy were prepared: 5-ethyl-5--(1-methylpropyl)-2-thiobarbituric acid (EMPTB), 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid (EMBTB), 5-allyl-5-(2-methylpropyl)-2-thiobarbituric acid (AMPTB), and 5-allyl-5-(1-cyclohexenyl)-2-thiobarbituric acid (ACHTB) by HCl precipitation from the corresponding sodium salts. These derivatives were used for our systematic investigations.

2-Thiobarbituric acid and its 1-methyl, 1,3-diethyl, 5-phenyl-1-methyl, 5-phenyl--1,3-diethyl, 5,5-diethyl-1-methyl, and 1,3,5,5-tetraethyl derivatives were prepared by means of a standardised method partially modified on the basis of literature data. These compounds were characterized on the strength of their melting points and microanalytical data.

We attempted to prepare the mixed complexes of pyridinocopper(II) and pyridinocobalt(II) but, as it turned out, no complexes were formed with 5-, N-, 5,N-, 5,N,N'-, or 5,5,N,N'-substituted 2-thiobarbituric acids, and only partially with 2--thiobarbituric acid. All the four 5,5- and the 5,5,N-substituted 2-thiobarbituric acids mentioned in Chapter 2.1. gave positive reactions. The reduced reactivity of the N,N'-substituted barbituric acid and thiobarbituric acid derivatives is proved unambiguously by the fact that complex formation fails to take place if there is no possibility of formation of a sulphohydryl group on the pyrimidine ring. At the same time this supports the hypothesis that the transition metal thiobarbiturate (TB) bond comes into being through the sulphur atom. The important role of the two substituents on C-5 (due to the electron releasing effect) is also obvious from our experiments. As a consequence of this effect and that of the tendency to conjugation, the proton of the strongly electron-attracting nitrogen becomes mobile and tends to form on C-2 a sulphohydryl group of enolic character ready to form complexes. These data were compared with pharmacological data and some interesting relations were found.

Pyridinocopper(II), -cobalt(II), -cadmium(II), and -nickel(II) EMPTB, EMBTB, AMPTB, and ACHTB mixed complexes were prepared in crystalline form, their formation conditions and some characteristic properties were described.

Microscopic photographs of the complexes mentioned above were presented, of which only pyridinocopper(II) EMPTB and AMPTB had previously been reported. (These, however, were obtained in a different way.) These photos furnished valuable data for the characterization of the complexes.

Our previous experiments showed that the pyridinocopper(II) thiobarbiturate mixed complex was analytically of great importance: consequently, this complex was examined in more detail.

The compositions of the pyridinocopper(II) EMPTB, EMBTB, AMPTB, and ACHTB complexes were determined. Their C, H, and N content was measured by microanalysis, their S content as sulphate after ignition in a BERTHELOT—MAHLER—KLÖCKER bomb, and their Cu content gravimetrically and complexometrically after ignition of the complex. The formula $Cu(II)(py)_2(TB)_2$ was assigned to the complexes.

At the time of the determination of the composition of copper(II)$(py)_2(TB)_2$ mixed complexes it was observed that these complexes decomposed and underwent a colour change when dried above 105 °C. This problem was studied in detail derivatographically; it was found that the examined complexes decomposed in a manner characteristic of mixed complexes. First they lose pyridine from the molecule; then the thiobarbiturate decomposes partially; finally copper(II) oxide remains. In the case of copper(II)$(py)_2(ACHTB)_2$ another decomposition mode was also observed, together with the loss of pyridine. According to our calculations it is probable that cyclohexene and decomposed thiobarbituric acid are lost from the complex.

The UV spectra of copper(II)$(py)_2(TB)_2$ mixed complexes were taken; these are to a great extent similar in the case of ligands with closely related structures. With EMPTB and EMBTB mixed complexes there is a characteristic sharp maximum at 288 nm, and others at 238—242 and 343 nm.

In the case of AMPTB mixed complexes there are absorption maxima at 260 and 344 nm, and for ACHTB mixed complexes also at 288 nm.

The spectrum of the pyridinocopper(II) complex of a TB containing a C-5 allyl is quite distinct from that of a TB containing a C-5 ethyl group.

Absorption and reflectance spectra of copper (II)$(py)_2(TB)_2$ and copper(II)$(TB)_2$ were measured in the region from 210 to 1250 nm. The results were interpreted on the basis of the ligand field theory. From the available data the conclusion could be drawn that the molecules in question are arranged in a square planar form. The electronic configuration of the copper in the complexes is as follows:

$$[Ar]d_\varepsilon^6,\ d_{xy}^2,\ d_{x^2-y^2}^2, \qquad 4s^2,\ 4p_x^2,\ 4p_y^2,\ 4d^1$$

In this case of a strong field, the ligands in the direction d_{x2-y2} are bonded so strongly and are so near the metal ion that they repel the electrons of the d_{xy} orbit thereby decreasing their energy. In accordance with the term splitting figure it can be established by means of the curve analysis method that is to say in the case of $Cu(TB)_2$ the centre of gravity of the main bands changes in the following order:

$$EMBTB < AMPTB < EMPTB < ACHTB$$

With complexes $Cu(II)(py)_2(TB)_2$, the order changes as follows:

$$AMPTB = EMPTB < ACHTB < EMBTB$$

In all cases

$$\lambda Cu(TB)_2 > \lambda Cu(II)(py)_2(TB)_2,$$

i. e. the bond character of compounds containing pyridine shifts significantly towards covalency.

Analysis of the absorption and reflectance spectra of cobalt(II) thiobarbiturate complexes showed that the cobalt atom was tetrahedrally coordinated and the bonds formed in the complex molecule had a high degree of covalent character.

The exploratory calculations on the basis of the TANABE—SUGANO energy matrices proved the same result. In accordance with these, the ν_1^* band ($=\delta$) can be expected at about 2000 nm. With all the compounds very close RACAH-B values were obtained and, consequently, similar β nephelauxetic Bo values ($\beta=B'$), where Bo$=$ $=971\ cm^{-1}$ (~ 0.60).

This finding was supported by our magnetic measurements, too. The complexes are paramagnetic with a value of about ~ 4.3 BM.

The analysis of IR spectra of the $Cu(II)(py)_2(TB)_2$ mixed complexes furnished further data concerning the structure of the complexes. The $\nu C=S$ band characteristic of TB appears in the spectrum of the compounds examined between 1150 and 1180 cm^{-1}. It is most intense in the case of thiobarbituric acid, due presumably to the ν—C—O contributions. In the mixed complexes the strong $\nu C=S$ bonds are absent; this is a proof that the bonding takes place through the sulphur. On the strength of our investigations and the analogy with the pyridinocopper(II) barbiturate mixed complex we have given the structural formula $Cu(II)(py)_2(TB)_2$ complexes.

The photometric determination of the thiobarbituric acid derivatives was first carried out on their pyridinocopper(II) mixed complexes. The reaction specificity of these from among other drugs, and the formation conditions of the mixed complex, were examined in water-pyridine solution. It turned out that under the given conditions only sulphadimidine gives an identical colour reaction among the drugs investigated. The determination can readily be carried out between pH 6.4 and 7.4. The intense green colour formed was measured in a 1 cm cell at 625 nm, after extraction with chloroform or benzene or after alkaline decomposition of the excess reagent. It is practical to measure samples of 5—25 mg. The determination is rapid and its accuracy lies within the usual $\pm 1\%$.

The photometric determination of thiobarbiturates as their copper(II) complexes was also elaborated. In this way adjustment of the pH is not required. From an investigation of the conditions of complex formation by means of the mole-ratio method, the complex appeared to be $Cu(II)(TB)_4$ in an abs. ethanolic medium. Working with 10—70 μg portions is the most convenient. The greenish yellow complex formed with copper(II) nitrate as reagent was measured at 360 nm in an 1 cm cell. The accuracy of the method is within 2%.

The photometric determination of thiobarbiturates as their cobalt(II) complexes was extended to other derivatives beside the two compounds described by HEISE and KIMBEL, and modified in several points. 5—10 mg of TB was measured out, allowed to stand for 2 hours with cobalt(II) chloride and solid potassium hydroxide. The extinction of the resulting green complex was determined photometrically at 625 nm in 1 cm cells. The appropriate reaction conditions and the dependence on temperature and time were established. The method could be well applied for determination of dry powders dispensed for injection in sealed ampoules (accuracy: within 2%).

Paper electrophoretic investigation of $Cu(II)(py)_2(TB)_2$ complexes showed that the electrophoretic mobility of these complexes was $6.17—7.46\times 10^{-5}$ cm^2/volt sec in a Kolthoff buffer of pH 9.7 (voltage: 200 V; current intensity: 24 mA; time: 4 hours in a wet cell; paper: W_1). This method was also suitable for detecting the thiobarbiturate parent compound in serum mercury (I) nitrate (1%) was used as developing reagent, after which the paper was treated with sodium hydroxide and then soaked in nitric acid. When dry, the thiobarbiturates and their pyridinocopper(II) complexes appeared as intense dark grey colours. Our thin layer chromatographic investigations were unsuccessful.

The results of the experiments are summarized in 23 Tables and 50 Figures.

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LIBEXIN

tablets — ANTITUSSIVE

COMPOSITION

3-(β,β-Diphenylethyl)-5-(β-piperidinoethyl)-1,2,4-oxadiazole hydrochloride 100 mg. per tablet

INDICATIONS

To relieve coughing of bronchial origin: Acute and chronic bronchitis, influenza (grippe), bronchopneumonia, cough due to tumour or some foreign body in the bronchi, bronchial asthma, emphysema, nocturnal coughing of cardiac failure, in preparation for instrumental examinations (bronchoscopy, bronchography).
To relieve coughing of pleural origin: Dry and exudative pleuritis, pleuropneumonia, pulmonary embolism, spontaneous pneumothorax, pleural interventions (surgical pneumothorax, pleural punction, pleural operations).

CONTRA-INDICATIONS

Pathological conditions with excessive bronchorrhea, first of all in post-operative condition (after inhalation narcosis).

DOSAGE

Average dose for adults is 3 to 4 times daily 1 tablet, in obstinate cases 2 tablets.
The usual dose for children is proportionally lower, depending on their age and body-weight, on the average 3 to 4 times daily ¼ to ½ tablets.
In premedication for bronchological interventions, Libexin should be combined with atropine, and given in doses of 0.9 to 3.8 mg. per kg. of body-weight one hour before the manipulation. Libexin has no unpleasant side-effects, and causes no habituation.

PACKING

20 tablets, 0.1 g.
200 tablets, 0.1 g.

CHINOIN
BUDAPEST, HUNGARY

MYELOBROMOL

tablets — CYTOSTATIC

COMPOSITION

1,6-Dibromo-1,6-dideoxy-D-mannitol	250 mg. per tablet

INDICATIONS

Early and advanced stages of chronic myeloid leukemia.

CONTRA-INDICATIONS

Hemorrhagic diatheses; concurrently with other cytotoxic therapies (X-ray, other cytostatics).

DOSAGE

Dose levels depend on the individual tolerance. Dosage should be adjusted to the actual hematological and clinical condition of the patient if the most favourable therapeutical result with minimum toxicity is aimed at.
The following dosage schedule is advised:
The *initial daily dose* should be 1 tablet (250 mg.) for a body-weight of 50 kg. ½ to 1½ tablets (125 to 375 mg.) for 25 and 75 kg., respectively. Upon this dosage as a rule the leukocytes gradually regress (sometimes preceded by a temporary rise) and clinical symptoms begin to improve. If possible this dose should be adhered to for a period of 3 to 6 weeks, depending on the patient's condition.When the leucocyte count reaches a value of below 20,000, maintenance therapy with ½ to 1 tablet every 2 to 3 days is instituted and put forth for 8 to 12 weeks. Frequent control of the blood-picture and the general condition remains further on necessary. By finding the adequate *maintenance dose* an early relapse can be prevented. As soon as the again-increasing leukocyte count evidences progression of the disease, it is indispensable to return to initial higher doses (½ to 1 to 1½ tablets daily) until the re-establishment of the clinical remission.
Complete hemogram including the platelet count has to be undertaken there times a week at the beginning of treatment, later on once a week.

SIDE-EFFECTS

Troublesome toxic effects affecting the gastrointestinal tract (anorexia, nausea, vomiting, diarrhoea) develop mainly as a result of overdosage. The administration of Myelobromol does not cause anemia, but may aggravate a casual thrombopenia, therefore at platelet counts under 100,000 treatment has to be suspended.

PACKING

10 tablets, 0.25 g.
100 tablets, 0.25 g.

CHINOIN
BUDAPEST, HUNGARY

NEVIGRAMON

capsules — CHEMOTHERAPEUTIC

COMPOSITION

1-Ethyl-7-methyl-1,8-naphthyridine-4-one-3-carboxylic acid 500 mg. per capsule

INDICATIONS

Primarily for the treatment of acute and chronic urinary tract infections caused by Gram-negative pathogens; E. coli, Salmonella or Shigella infections of the gastrointestinal tract; brucellosis.

CONTRA-INDICATIONS

Epilepsy, hepatic and renal impairment. In cases of respiratory depression and in the first third of gravidity dosage should be particularly cautious.

DOSAGE

Average daily dose for adults four times daily 2 capsules (4.0 g.) for at least 7 days, and if necessary, for a longer period of time. Children receive proportionally less in accordance with their age and body-weight ;on the average 60 mg. per kg. of body-weight, in four equal doses.

SIDE-EFFECTS

Undesirable side-effects (nausea, vomiting, diarhrhoea, headache, vertigo), as well as skin symptoms (erythema, urticaria) due to sensitisation occur but seldom. Reactions of the central nervous system (headache, dizziness, visual disturbances, depression or excitement) are occasional.

PACKING

56 capsules, 0.5 g.

CHINOIN
BUDAPEST, HUNGARY

NO-SPA

tablets and ampoules — SPASMOLYTIC

COMPOSITION

6,7,3′,4′-Tetraethoxy-1-benzal-1,2,3,4-tetrahydroisoquinoline hydrochloride (Drotaverine hydrochloride) 0.4 g. per tablet and ampoule (2 ml.)

INDICATIONS

In general, to abolish or prevent pain and dysfunction caused by smooth muscle spasm. Biliary and renal colics, spasms associated with cholecystopathy, cholelithiasis, cholecystitis, nephrolithiasis, pyelitis, cystitis, as well as spasms elicited by instrumental examination. Spastic conditions of the gastrointestinal system; gastric and duodenal ulcer, laryngo-, cardio- and pylorospasm, spastic constipation, colitis, proctitis, tenesmus. In acute blood-pressure fluctuations of hypertensives, administered in conjunction with antihypertensive drugs. Coronary and cerebral vascular spasm; intermittent claudication; dysmenorrhoea; to reduce the irritability of the pregnant uterus; in spasm of the uterinal orifice during delivery, protracted first stage of labour, after-pains, threatened abortion, etc.; in case of postoperative wind colics.

DOSAGE

The average dose for adults is daily three times 1 to 2 tablets or daily once to three times 2 to 4 ml. injected subcutaneously or intramuscularly. In emergency, e.g. acute stone colic, 2 to 4 ml. may be given by slow intravenous injection. In case of peripheral arteriál spasm or obstruction No-Spa may be injected intraarterially. Children should be given smaller doses according to age and body-weight. Small children should receive once or twice daily ¼ to ½ tablet, older children ½ to 1 tablet daily.
In peptic ulcer it is expedient to combine No-Spa with atropine or atropine-like compounds.

PACKING

20 tablets, 0.04 g.
100 tablets, 0.04 g.
1000 tablets, 0.04 g.
5 ampoules, 0.04 g. (2 ml.)
50 ampoules, 0.04 g. (2 ml.)

CHINOIN
BUDAPEST, HUNGARY

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